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Absorption and translocation of 2, 4-dichlorophenoxyacetic acid and of radioactive phosphorus

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NOTE TO USERS

ABSORPTION AND TRANSLOCATION OF 2,4-DICHLOROPHENOXYACETIC ACID AND OF RADIOACTIVE PHOSPHORUS

by

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INTRODUCTION

The past decade has seen the development and widespread use of systemic herbicides, and systemic insecticides and fungicides are now being developed. These compounds may be applied to a portion of the plant and be transported, apparently by the normal translocating mechanism, into all its tissues and organs. The translocation of these substances is a prerequisite to the desired results in most instances. The use of these compounds has placed renewed emphasis on the importance of translocation in plants, and has increased the efforts of plant physiologists to elucidate the mechanism involved.

The compound, 2,4-dichlorophenoxyacetic acid (2,4-D), is the most widely used of the systemic herbicides. Because of its effects on growth the presence of 2,4-D is easily detected in many plants. The growth of these plants after treatment may be used as a measure of the 2,4-D present. Swanson and Whitney (1953) have shown that labeled phosphate applied to plant leaves was absorbed and translocated from them in the phloem. The amount of P^{32} in a plant may be measured by the quantity of beta radiation emitted. The ease with which P^{32} can be detected and the fact that it may be translocated in the phloem make it a good material to compare with 2,4-D in translocation studies.

Systemic agricultural chemicals are commonly applied to the foliage of plants. Absorption of these materials is the first step in their distribution through the plant. The distance into the leaf cell is short, but the direct route is across cuticle, cell wall and cell membranes. This is a complex pathway, and the process of absorption is incompletely understood. It is necessary to separate absorption from translocation before the latter can be studied in detail.

The objectives of the investigations reported in this thesis were: to separate absorption of 2,4-D and labeled phosphate from translocation in order that these two processes might be studied independently; to study factors affecting the absorption and translocation of these two materials; to compare the behavior of labeled phosphate with 2,4-D.

REVIEW OF LITERATURE

Absorption

Absorption of 2,4-D by leaves

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is often applied to plant foliage. Before a foliar application of 2,4-D affects the growth of plants, it must be absorbed into the plant tissue. Several factors have been found to influence the quantity absorbed and the rate of absorption.

It was early recognized that the ester forms of 2,4-D are more toxic than salt forms at the same rate. Hitchcock and Zimmerman (1948) cautioned that only minimum dosages of esters should be used on crop plants. Derscheid (1948) summarized the work of a number of authors, and concluded that less ester of 2,4-D than the amine or sodium salt should be used on small grain. Their conclusions agree with the work of Beevers *et al*. (1952) on the absorption of the esters of biologically active weak acids. They found that the diethyl ester of malonic acid was much more effective in inhibiting respiration *in vivo* than was the acid or its sodium salt. They concluded that the differences were due to the rate of absorption of the two forms into cells.

The addition of Carbowax to sprays of 2,4-D acid increased the toxicity to kidney beans (Ennis and Boyd, 1946). Hitchcock and Zimmerman (1948) used "Tween 20" and several other wetting agents with Na-2,4-D sprays. They found that the wetting agents, as well as several other compounds, increased the toxicity of 2,4-D to tomatoes. Staniforth and Loomis (1949) reported that small amounts of certain surface-acting compounds added to sodium and ammonium 2,4-D sprays applied to flax and corn greatly reduced the yields compared with plots treated with the same rate of 2,4-D alone. The same effect was noted in greenhouse experiments with soybeans. Several diverse types of commercial wetting agents gave this response. Part of the explanation appeared to lie in decreased surface tension of the sprays, but the relationship was not a simple one. Even though the surface tension was not lowered significantly by more than 0.05 per cent of a sodium lauryl sulfate type of wetting agent, increased response to 2,4-D sprays was obtained with additions up to 2.0 per cent. Hauser (1955) found that Na-2,4-D in solution did not enter soybean leaves during a 60 minute exposure period in sufficient quantity to reduce growth. But when surface active agents were added significant absorption occurred in 30 minutes. With a solution of 750 ppm. of the amine, the exposure time required to cause significant reduction of new growth was four minutes with wetting agents and seven minutes

without them. Although ester formulations are absorbed much more rapidly than the salts of 2,4-D, the rate of absorption of the isopropyl ester of 2,4-D was increased by two of three surface activants used.

The general effects of temperature on the toxicity of 2,4-D were noted by early investigators (Marth and Davis, 1945; Kelly, 1949). Hauser (1955) states that as the temperature during a 30 minute exposure was increased from 37 to 81° F., more 2,4-D was absorbed. This was in agreement with the earlier findings of Bryan et al. (1950).

Hauser (1955) noted that the supply of carbohydrates in the leaf did not affect the quantity of 2,4-D absorbed, but the toxicity of the applied solution decreased with a pH above 7. Hamner et al. (1947) found that the activity of the sodium salt of 2,4-D was increased at low pH's. The maximum toxicity to beans occurred at a pH of 2 to 2.2.

The absorption of radioactive phosphorus by leaves

Robertson (1951) reviewed the literature on the absorption of inorganic nutrients by plants. All the problems involved in the uptake of ions by leaves were not considered. The work he reviewed was mostly with plant roots or excised tissues.

Colwell (1942) reported that a portion of the P^{32}, K^{42},

Ca^{45}, and Cs^{137} applied to the surface of leaves was absorbed by them. Swanson and Whitney (1953) studied the absorption of radiophosphate by bean leaves. They found that the amount of P^{32} absorbed was greater as the pH was decreased from 7 to 2. Solutions with a pH of less than 3 to 3.5 caused necrosis of the leaves. The authors attributed these effects of pH to a lowered dissociation of the phosphoric acid and a possible direct effect on the permeability of the leaves.

Eroding the leaf surface with a fine abrasive increased the absorption of P^{32}, but the one wetting agent tried reduced absorption (Swanson and Whitney, 1953). It was thought that the wetting agent, "Tween 80" (a polyethylene sorbitan monoleate), formed a complex with the phosphate ions.

Translocation

Tissues involved

Many facts are known concerning the movement of solutes in plants but much remains to be learned concerning the process. Only in recent years have the tissues involved been definitely established. Before 1920 it was commonly accepted among botanists that upward translocation of solutes, both organic and inorganic, occurred in the xylem, while the principal pathway of downward translocation was the phloem.

These conclusions were based on ringing experiments such as those of Hartig (1858) who showed that the starch in the roots of oaks disappeared after the bark was removed in a ring around the trunk. At the same time the starch did not disappear from the roots of cut trees, thus leading Hartig to conclude that the starch in the roots of the ringed trees had moved through the xylem to the tops. It is commonly observed that the sap exuded from cut xylem contains appreciable quantities of mineral salts and at certain seasons considerable sugar also. Jones et al. (1903) reported as much as 8 per cent sugar in the sap of sugar maples in the spring.

In 1920 two papers were published which drew diametrically opposite conclusions concerning the pathway of organic solute movement. Birch-Hirschfeld (1920) suggested that her experiments indicated that both upward and downward movement took place in the xylem. From the results of his first ringing experiments Curtis (1920a) concluded that both upward and downward translocation of organic compounds occurred in the phloem. He showed in this and later research (Curtis 1920b, 1923) that severing the phloem connections of budding woody stems greatly reduced the growth of these stems. This was undoubtedly a reflection of the curtailment of the supply of reserve food materials from storage tissue below the ring. Later Curtis (1925) removed the wood from sumac and mock

orange stems while leaving the bark intact except for vertical slits necessary to cut out the wood. The stem was supported and the area where the wood was removed enclosed in a tube of water. This complete removal of the xylem had little effect on the upward movement of organic solutes to the stem above. Flushing out the water jackets once each day with distilled water gave similar results. When the phloem was removed instead of the xylem, translocation of organic solutes was apparently stopped. All the stems were defoliated at the start of the experiments, and the amount of material translocated was measured in terms of rate of elongation, dry weight and carbohydrate content of the treated stems compared with checks.

Mason and Maskell (1928) concluded that ringing of cotton stems caused an accumulation of carbohydrate above the ring. When the bark was left intact, but separated from the wood by a strip of waxed paper, transport of carbohydrates out of the leaves above took place at a nearly normal rate. Later experiments by Maskell and Mason (1930) led them to conclude that nitrogen moved out of cotton leaves through the phloem. They found that nitrogen accumulated above a ring on the stem, which indicated that nitrogen could move upward from the roots through the xylem. Clements and Engard (1938) also concluded that the upward movement of mineral salts was not affected by ringing woody stems.

Curtis (1929) reported that when bean petioles were chilled to 1.4-6° C. the removal of carbohydrates from the leaves was stopped or greatly retarded. Curtis and Herty (1936) found a direct relation between the rate of carbohydrate transport through bean petioles and the temperature of the petioles between 4.5 and 24° C. These experiments indicate that living tissues are actively involved in the transport of carbohydrates.

The question of the tissues involved in the movement of organic and inorganic solutes in plants was not entirely settled until radioisotopes became available for biological studies. Stout and Hoagland (1939) used isotopes of Na, K, P and Br to study the upward movement of these elements. Sections of bark and wood of cotton, geranium and willow were separated with waxed paper, and the element in question supplied to the roots in culture solution. The results showed that upward movement of mineral salts studied may occur in the xylem, but the possibility of such movement in the phloem is not completely excluded. Results of Biddulph's (1940) work with phosphorus are in agreement. It is now generally accepted that inorganic forms of mineral elements move from the roots in the xylem, but organic forms move largely in the phloem (Loomis, 1935; Meyer and Anderson, 1952).

General aspects of solute translocation in the phloem

From the extensive experiments that have been directed toward elucidating solute movement in plants, certain facts are apparent. A general summary of these facts will be useful before we consider hypothetical mechanisms.

The movement of organic solutes through the phloem is rapid. Crafts and Lorenz (1944) calculated that if the organic compounds used in the growth of certain cucurbit fruits moved as a 10 per cent solution, the rate of movement would be about 110 cm. per hour. Using C^{13} to label the photosynthate in sugar cane, Hartt and Burr (1950) calculated the minimum rate to be 43 cm. per hour. This estimate included the time required for the $C^{13}O_2$ to be fixed, transported to the roots and released as $C^{13}O_2$ by respiration and was, therefore, slower than the actual rate of transport. From activity curves at five and 20 minutes Vernon and Aronoff (1952) calculated a rate of 1.4 cm. per minute for the translocation of C^{14} labeled photosynthate in soybeans. Day (1952), from several experiments with 2,4-D, estimated the rate of movement of this herbicide to be approximately 50 cm. per hour. Day used stem curvature as an indication of the presence of 2,4-D. Biddulph and Markle (1944) estimated the rate of translocation of P^{32} in the phloem to be in excess

of 21 cm. per hour.

In many of these estimates assumptions have been made concerning the time required for absorption or response, the volume of the sieve lumena or other factors, leaving some uncertainty of the accuracy of the results. It is apparent, however, that rapid movement of solutes in the phloem may occur.

Large quantities of organic compounds are transported in plants, often through stems of small diameter. The smaller total diameter of the phloem elements often makes the feat appear spectacular. Clements (1940) worked with an extreme example, the sausage tree (Kigela africana). He estimated that as much as 32.6 g. of organic material moved through one slender stem in a 24-hour period. Calculations of a similar nature, made by Crafts and Lorenz (1944) on the growth of pumpkins, estimated an average hourly increase in dry weight per fruit of 0.61 g. over a 33-day period.

Curtis (1929) called attention to the fact that active living tissue is necessary for the transport of organic compounds. When he reduced the temperature of bean leaf petioles from 24 to 4-6° C. or enclosed them in tubes of N_2 under slight pressure, transport of carbohydrates out of the leaves was greatly reduced or stopped. Work by Curtis and Herty (1936) showed that chilling bean petioles to temperatures of 0.5 to 4.5° C. greatly reduced the transport of carbohydrates through

them but did not stop translocation. Temperatures of 7-11° C. allowed for more transport than the lower temperatures but not as much as temperatures of 17-24° C. Mason and Phillis (1936) found a supply of O_2 to the transporting tissues was necessary for the translocation of both carbohydrates and nitrogenous compounds from the leaves of cotton plants. Hewett and Curtis (1948) found that the rate of translocation increased in milkweed, bean, and tomato reached a maximum and then declined in the range of 4-40° C. The maximum rate of translocation occurred at a higher temperature for milkweed than for bean or tomato. Swanson and Bŏhning (1951) measured translocation through the petioles of darkened bean plants. One primary leaf was dipped into a sucrose solution and the elongation of the terminal bud measured. The petiole of the supplying leaf was jacketed and the temperature varied, while the temperature of the remainder of the plant was held constant. Translocation was reduced as much as 50 and 100 per cent at temperatures of 5-7.5° and 40-42° C., respectively, when compared to controls at 20° C. Maximum translocation occurred at temperatures between 20 and 30° C. Later Bŏhning *et al*. (1952) used the same technique, except that the experimental leaf was allowed to perform photosynthesis to supply carbohydrates. The temperature coefficient for the translocation of this photosynthate was higher than for the translocation of the supplied carbohydrate. Bŏhning *et al*. (1953)

found the optimum temperature for the translocation of carbohydrate in beans to be approximately 24° C. The temperature coefficient in the range of 12-24° C. was about 1.5.

Went and Hull (1949) and Hull (1952) have reported experiments which were claimed to give a temperature coefficient of less than one for the translocation of carbohydrate in the range of 1-23° C. These authors used Went's indirect "bleedometer" technique to measure the effect of a temperature jacket around part of the transporting tissue. Their measurements of actual translocation showed a positive response to temperature.

Rabideau and Burr (1945) blocked the transport of C^{13} labeled photosynthate by killing a section of stem. Weaver and DeRose (1946) and Day (1950) have made similar observations concerning the effect of killing a section of stem on the translocation of foliar applications of 2,4-D. One may conclude that living tissue is necessary for the translocation of organic compounds.

Phillis and Mason (1933) reported that sucrose was accumulated in the phloem of cotton leaves against a concentration gradient. When sugar beet leaves were darkened, Leonard (1939) found that the sugars in them were transported out and into the root until the leaves were starved. Young, growing leaves obtained sugar from the root, but after they had grown to about one fourth mature size, darkened leaves died

of starvation although they were attached to the root which abounded in sucrose. Wanner (1953) analyzed the sugars contained in the phloem sap and leaf parenchyma of black locust, separating them by chromatographic methods. While leaves contained equal amounts of dextrose and levulose along with greater quantities of sucrose, the phloem sap contained only sucrose and at a concentration 20 times that in the leaf. The developing corn ear is capable of marked polarization of the available sugar in the plant (Loomis, 1945). After the ear had been pollinated and had developed for four to eight days, the leaves from the fruiting stalk could be removed and the ear would still develop normally on carbohydrates from sucker leaves as much as eight feet away.

Phillis and Mason (1936) deduced from analyses of the carbohydrate and nitrogen contents of ringed and unringed cotton plants that nitrogen was moving up the plant stem at the same time that carbohydrates were moving downward. Palmquist (1938) found evidence that sugar and the dye fluorescein could move simultaneously in opposite directions in the same region of phloem. Chen (1951) was able to show that C^{14} labeled photosynthate and P^{32} moved through the phloem of geranium simultaneously in opposite directions, at least over a short distance and at a relatively slow rate. Whether or not simultaneous movement in opposite directions may take place in the same sieve tube has important implications in

any consideration of the mechanism involved in phloem transport. Unfortunately no one has yet devised an experiment which shows whether or not this phenomenon can occur.

Proposed mechanisms

Three theories have been advanced to explain the translocation of solutes through the phloem of plants. In 1936 Mason and Phillis set up their theory of "activated diffusion" of solute molecules. Their experiments showed movement on concentration gradients, but at rates greatly in excess of those expected of diffusion alone.

Münch (Crafts, 1931) based a mass flow hypothesis on sound physical principles, but failed to take thorough consideration of the tissues involved. The motive power for this mass flow of sap through sieve tubes was proposed to be an osmotically generated turgor pressure gradient. Münch considered that sugar produced by photosynthesis increases the osmotic pressure in leaf parenchyma, resulting in a withdrawal of water from the xylem and creating a high turgor pressure in these cells. This pressure then was thought by him to cause a flow of solution through the plasmodesms from cell to cell and into the phloem. There the sugar increases the osmotic pressure and additional water is then withdrawn from the xylem, increasing the hydrostatic pressure. This pressure

is assumed to result in a flow along the strands of sieve tubes to the stem and roots where food materials are removed and water is discharged into the xylem. The flow so created is maintained by a continuous re-supply of sugar at the leaf (the source) and a continuous utilization or storage of the solutes at the receiving end (the sink). In the picture presented above, any source of solutes could drive the solution toward any sink that removes these solutes and reduces the osmotic pressure.

Münch first presented this hypothesis to explain his observations on the exudation of sap from the phloem of woody plants. Crafts (1931, 1932) found this hypothesis compatible with the results of his experiments on exudation from the cut phloem of cucurbit stems. Since this time Crafts has been the principal exponent of this hypothesis and has termed it the pressure flow hypothesis. He early recognized a major weakness, the fact that phloem elements are not continuous open conduits as appears necessary for the physical model, but are abutting cells with end walls and protoplasm. The end walls form the perforated sieve plates. The tiny holes in these plates are commonly filled with cytoplasm or slime plugs. Crafts (1932) first attempted to resolve this difficulty by theorizing that a portion of the solute movement took place through the cell walls. When he compared the force calculated by him to be necessary to account for the observed

rate flow of exudate from the sieve tubes with the osmotic pressure of the exudate, the force was much greater than the turgor pressure that could be developed by the solution. Later Crafts (1938) changed his views and proposed that the protoplasm in the sieve tubes is highly permeable to solutes. Curtis and Asai (1939) pointed out that if the sieve tubes are permeable to their contents there would be lateral leakage. They found that phloem exudate would not plasmolyze their respective parenchyma cells. This they interpreted to mean that the sieve tubes are not completely permeable to their contents.

Currier et al. (1955) were able to plasmolyze and deplasmolyze mature sieve elements of 23 species, often in repeated cycles. There were differences between species and not all species could be plasmolyzed at all seasons of the year. Unless the cells could be deplasmolyzed they were not considered plasmolyzable. Mature sieve elements that could be plasmolyzed did not show protoplasmic streaming, accumulate neutral red or produce formazan when treated with tetrazolium, reactions which are usually interpreted to indicate live plant tissue. The wide spectrum of species that were found to have plasmolyzable phloem elements leaves little doubt that mature sieve elements are differentially permeable. The physical model for pressure flow does not work if one differentially permeable membrane is inserted between the source

and the sink.

There are other weaknesses in the pressure flow hypothesis. Calculations indicate that the pressures developed are inadequate to account for the observed rates of movement (Crafts, 1932). Bonner (1944) and Swanson and Whitney (1953) observed independent rates of movement for several different materials which were investigated. The pressure flow hypothesis does not account for movement at independent rates or for the possibility of simultaneous movement in opposite directions in the same phloem area. The direction of movement is often not from a region of higher to a region of lower solute concentration (Curtis and Scofield, 1933; Loomis, 1945). Crafts (1951) has suggested ways in which the hypothesis could surmount some of these difficulties.

Early botanists suggested that solutes are transported in streaming protoplasm. Curtis (1935) and his coworkers have been the principal exponents of this hypothesis. Proof for this hypothesis lies in the retardation or checking of translocation by conditions which are known to inhibit protoplasmic streaming. Curtis (1935) has explored the evidence for this hypothesis, and evidence since this publication has been largely of the same nature. The mechanism was proposed by Curtis (1935) to function as follows: the moving solutes are carried through the cell by protoplasmic streaming and diffuse through the end walls into the adjacent cell. In

this manner the solutes are carried through the phloem strands.

This hypothesis also has weaknesses. No one has observed protoplasmic streaming in mature sieve tubes of higher terrestrial plants. The rate of movement by this mechanism also seems inadequate.

It is apparent that the translocation of solutes through phloem only occurs in living tissues and at rapid rates. The mechanism involved is obscure.

The translocation of 2,4-D

Before 2,4-D was generally available for research, Ferri (1945) reported that this compound and certain other growth regulators moved up plants in the xylem when applied to the soil around the test plants. This movement was not dependent upon living cells. Mitchell and Brown (1946) demonstrated that the stimulus resulting from the treatment of bean foliage with 2,4-D moved out of the leaves through living tissue. The movement of this stimulus was correlated with the movement of organic foods. Translocation of the 2,4-D stimulus did not occur from leaves that had a low sugar content, but was rapid from leaves exposed to light and CO_2 adequate for photosynthesis. When 2,4-D was applied to bean roots the stimulus produced was transported upward through non-living cells of the stem, indicating movement through the xylem.

Weaver and DeRose (1946) obtained similar results with the movement of 2,4-D stimulus in bean plants. Their work indicated upward movement of root applications of 2,4-D through the xylem, with subsequent accumulation in the phloem. When defoliated bean plants were treated on the stems with 2,4-D only very localized bending occurred, while bean plants with the leaves left on gave much more widespread response to 2,4-D. Clipping off most of the leaf before treatment almost stopped outward movement of 2,4-D. The conclusions drawn by these early papers, that 2,4-D moves upward through the xylem when absorbed by the roots and up and down in the plant through the phloem when applied to the foliage, have been verified by much research since that time.

Rohrbaugh and Rice (1949) found no evidence that sodium 2,4-D was translocated from destarched bean plants in the dark until sugar was added to the leaves. Sugar was supplied to destarched bean plants by dipping the intact leaves in a sugar solution or by placing the cut tips of leaf blades in a 10 per cent sugar solution. Fructose and glucose appeared more effective than sucrose in causing the transport of 2,4-D.

Linder et al. (1949), working with three esters of 2,4-D and one of 2,4,5-T, reported that translocation of these compounds was related to conditions favoring carbohydrate movement. Application of these herbicides in Varsol, kerosene or motor oil gave similar results. The number of carbon

atoms in the alcohol chain of the ester did not change this relationship.

Weintraub and Brown (1950) investigated the relation between carbohydrate movement and the translocation of the acid of 2,4-D, its morpholine salt and six other growth regulators. None of these compounds was transported into the stem when applied to bean leaves devoid of carbohydrates. Translocation of all of these growth regulators could be brought about by providing an external supply of sugar to the treated leaves. A variety of sugars (sucrose, glucose, fructose, galactose, and maltose) were able to induce translocation. From the non-specificity for either growth regulator or sugar, it appeared that there was no specific combination between the two. Rather, movement of the growth regulator seemed to require only that the mechanism for transport of organic materials be activated.

Penfound and Minyard (1947) doubted that all movement of 2,4-D was correlated with the transport of food materials. When four drops of a 1000 ppm. solution of the butyl ester of 2,4-D in kerosene was applied to water hyacinth, injury was greater when the plants were in sunlight than when in shade. With the same treatment beans showed a similar bending and necrosis whether in darkness, diffuse light, or full sunlight. Rice and Rohrbaugh (1953) applied 2,4-D, carried by 50 µl. of kerosene, in aluminum rings sealed to bean

leaves with agar. This treatment caused a comparable amount of stem curvature and subsequent reduction in growth whether applied to carbohydrate depleted plants or bean plants in full sunlight. Control plants treated with kerosene alone had kerosene in the petioles and stems, as shown by a stain specific for petroleum oils. When 2,4-D was labeled with C^{14} it was found to move with the oil at a rate of about 4 cm. per hour. The authors concluded that the oil crept through the plant tissues and carried the 2,4-D with it.

Lowering the temperature below 20-25° C. was reported to reduce the speed of response of plants to 2,4-D (Marth and Davis, 1945; Kelly, 1949). Day (1950) reported that reducing the temperature of a section of stem reduced the rate of transport of 2,4-D in bean plants, even when this section was out of the path of movement. The rate of translocation in treated soybeans was greatly reduced when the temperature of the plant was reduced from 30 to 10-15° C. (Young, 1953).

Dhillon and Lucas (1950) were able to detect a material causing 2,4-D-like responses in water and ether extracts of plants after 2,4-D had been applied to the roots. Holley et al. (1950), using C^{14} labeled 2,4-D, found that over one-half of the active material they were able to isolate from treated beans was not 2,4-D. Fang et al. (1951) treated primary bean leaves with labeled 2,4-D. The greatest amount of activity was accumulated in the stem and first internode. Jaworski

and Butts (1952) used paper chromatography to separate the three major active components isolated from bean plants treated with labeled 2,4-D. One was 2,4-D and the other two were not identified. There was an inverse relationship between the amount of 2,4-D and the amount of unknowns in the stem, suggesting that the unknowns were formed by a reversible reaction of 2,4-D with other molecules. In corn and wheat a major portion of the activity applied as labeled 2,4-D was found in an unknown compound with a different R_f value than those found in beans (Fang and Butts, 1954). These authors postulated a block in the intercalary meristems of these monocots to explain slower translocation than was observed in bean plants. Under identical conditions there was no difference in the rate of formation of the 2,4-D complex in etiolated and normal bean seedlings (Jaworski et al., 1955). Whether or not the formation of a complex is necessary for the translocation of 2,4-D remains to be determined.

The translocation of radiophosphorus

Radioactive phosphate has proved to be an excellent tracer material for the study of solute translocation in plants. It has been used to study both movement in the xylem and the phloem. When phosphate is supplied to the roots, it may move upward in the xylem (Gustafson and Darken, 1937;

Stout and Hoagland, 1939; Biddulph, 1940; Rabideau and Burr, 1945).

Foliar applications of labeled phosphate may cause it to move out of the treated leaf through the phloem. Biddulph (1941) developed the "flap technique" for getting $P^{32}O_4^{\equiv}$ into leaves. When this technique was used, applied phosphate moved up and down the stems through the phloem (Biddulph and Markle, 1944). Colwell (1942) placed labeled phosphate solution in squash leaves by vacuum infiltration and by simply applying the solution to the leaf surfaces. When the phosphate solution was applied to the surfaces or infiltrated into leaves not under stress for water, the P^{32} would not move out of the treated leaves when sections of the petioles were steamed. Swanson and Whitney (1953) applied a small droplet of labeled phosphate solution to primary bean leaves with a micropipette. These foliar applications of phosphate would not move out of the leaf through a 5 mm. section of steamed petiole. They jacketed the petioles of treated leaves in order to control the temperature of them. Low temperatures markedly inhibited translocation, inhibition amounting to 85 per cent or more at a temperature of approximately 5° C. For four-hour translocation periods the optimum temperature was usually in the neighborhood of 30° C., with higher temperatures causing a decline in rate. Localized control of the temperature of the stem had a similar effect on the upward

and downward movement of foliar applications of P^{32}. On the other hand, localized low stem temperatures had no effect on the rate of upward movement of P^{32} supplied to the roots of bean plants. The temperature of the jacketed petiole had an effect on the amount of phosphorus accumulated in it. The optimum temperature for accumulation was near 40° C., higher than that for translocation.

Biddulph and Markle (1944) calculated the rate of movement of P^{32} through the phloem to be in excess of 21 cm. per hour. Movement through the phloem has been correlated with food movement in squash (Colwell, 1942).

METHODS AND MATERIALS

Studies with 2,4-D

General methods

Soybean was selected as the test plant because it is easily grown, susceptible to 2,4-D, and the primary leaves are convenient for the application of treatments. Five or six seeds of the Hawkeye variety were planted in four-inch pots of mixed greenhouse soil. At the time the unifoliate leaves were expanding each pot was thinned to two uniform plants. Each replication was carefully selected to give maximum uniformity of experimental material. The plants, grown in the greenhouse, were treated when they had reached the stage that the unifoliate leaves were fully expanded but the trifoliates were rolled tightly in the bud. In most experiments a solution of Baker's sodium 2,4-D monohydrate was applied by dipping the distal two-thirds of one unifoliate and carefully shaking off the excess solution. The standard treatment, selected after preliminary experiments, was 750 ppm. of 2,4-D plus 0.1 per cent of Triton B-1956 as a wetting agent. This wetting agent was known to be relatively non-phytotoxic, but in all experiments the controls were dipped in 0.1 per cent wetting agent. When sucrose was applied at

the same time as 2,4-D, the two compounds were placed in one solution. A 5 per cent sucrose solution was used in all experiments.

Temperature chambers were obtained by placing germinators containing thermostatically controlled heaters in a refrigerated room maintained at a temperature below those desired. A small electric fan was placed in each germinator to keep the air moving and to prevent a temperature stratification. In this way the temperature in the chamber was maintained with a variation of $\pm$ 1° C.

When plants depleted of carbohydrates were desired, light was excluded from the plants by placing them in a tightly closed box in the greenhouse. Plants were brought into the light for treatment, then quickly replaced if they were to be held in the dark for another 24 hours.

The effects of 2,4-D were measured by taking the green weight of the trifoliate growth 10 to 14 days after treatment. Visual symptoms of 2,4-D injury were noted also.

Methods for specific experiments

In order to study the effects of temperature on the absorption of 2,4-D, soybeans were treated and immediately placed in the proper temperature chambers. At the end of two hours, as the plants were taken from the chambers, the

excess 2,4-D was washed from each treated leaf by first dipping it into four beakers of Dreft solution and then four beakers of tap water. Preliminary experiments showed that this washing removed all detectable 2,4-D from the leaf.

The effect of sucrose on the absorption of 2,4-D by leaves depleted of carbohydrates was studied by dipping them in a solution containing 750 ppm. of 2,4-D and 5 per cent sucrose. The plants were then returned to the dark for 24 hours. The treated leaves were washed by the method previously described at the time the experimental plants were returned to the light. It was assumed that the 2,4-D translocated from these leaves after the plants were returned to the light was proportional to the amount absorbed. New growth was used to measure the amount of 2,4-D translocated.

In the translocation experiments soybean plants were treated by dipping and left for two hours at a temperature of approximately 30° C. to obtain uniform penetration of the herbicide into all treated leaves. The fact that all plants were at the same temperature in a given experiment during absorption should be stressed. Experiments with the rate of absorption showed that no detectable amount of 2,4-D moved from treated leaves in this time (Table 6). Each treated leaf was tagged by placing a loop of cotton string around the petiole. After this absorption interval each treated leaf was washed as described above to remove any unabsorbed

herbicide. The plants were placed in the desired temperature chambers for a period varying in length from one to 25 hours. Various combinations of 5, 15, 25 and 30° C. were the temperatures used. The details of each experiment are presented with the results. As the plants were taken from the temperature chambers, each treated leaf and petiole were removed to prevent further translocation. The plants were returned to the greenhouse and allowed to grow for 10 to 14 days. Results were measured in terms of the new growth on the plants.

Plants with the leaves depleted of carbohydrates were used in studies of the effects of sugar on the transport of 2,4-D. After treatment, one group of plants was allowed to remain in full sunlight and the other was returned to the dark chamber for 24 hours. At the end of this period all treated leaves and petioles were removed and the plants placed in the greenhouse to grow before harvesting and weighing.

The oil used as a carrier in some experiments was a highly refined non-phytotoxic number 10 Base oil obtained from the Standard Oil Co. of Indiana. The effect of this carrier on the movement of 2,4-D from carbohydrate depleted leaves was studied. Two hundred micrograms of the propyleneglycolbutyletherester of 2,4-D in 50 µl. of the Base oil was placed on a leaf in an aluminum ring. The ring, one-half inch in inside diameter, was sealed to the leaf with agar as described by Swanson and Whitney (1953). The ring prevented

the oil from creeping over the surface of the plant. Care was taken to see that none of the rings leaked oil. All plants were kept in the dark chamber for 24 hours, then the treated leaves and petioles were removed as the plants were returned to the light. The check plants were treated with the carrier oil only.

Soybeans were germinated in moist sand and the seedlings suspended in quart jars of nutrient solution to determine the effect of temperature on absorption and translocation of 2,4-D by roots. The solution was changed every five days. In order to lower the temperature of the solution around one set of plant roots, the jars were placed in a metal container and cold tap water allowed to flow around them. The temperature of the nutrient solution was lowered to 13° C. in this manner. The other set of plant roots was maintained at 23° C. by placing them in a container of water at room temperature, and controlling the temperature of the greenhouse to keep the water at this temperature. The plant roots were held for a period of 24 hours at either 13° or 23° C. with 45 or 135 ppm. of Na-2,4-D.

Studies with Radioactive Phosphorus

General methods

Soybeans, grown and selected as described earlier, and sugar beet leaves were used in the P^{32} experiments. The sugar beets were grown through the summer near Ames. In the fall selected plants with large roots were transplanted to 12-inch pots and brought into the greenhouse. Leaves for experimental purposes were cut from the plants, the petioles quickly placed in water, and about a two-inch section cut from the petioles under water. This method produced leaves that would remain turgid for a week or more, unless subjected to very high rates of transpiration.

The P^{32}, a high specific activity solution of the phosphate ion in weak HCl, was obtained from the Oak Ridge National Laboratory. One microcurie in 10 µl. of solution was the standard rate of application. The material was applied to the center of one unifoliate soybean leaf with a micropipette. In the studies with beet leaves about 100 µl. of solution containing 5 µc. of P^{32} and 0.2 per cent Triton X-100 as a wetting agent was spread evenly on each leaf surface with a soft brush.

In the experiments with soybeans the tops of the experimental plants were removed and dried for 24 hours at 65° C.

The two plants in each pot were combined for analysis and ground in a Wiley mill to pass a 20 mesh screen. The dried material was pressed into a briquet by the method of MacKenzie and Dean (1950) as modified by McCants (1955). This method required 1.5 g. of material to give a sample of infinite thickness. In this research the two plants used as a unit did not weigh 1.5 g. Enough untreated soybean material was added to bring the weight of each sample to 1.5 g., then thoroughly mixed with the active sample. Each briquet was counted with a lead-shielded, thin mica, end window geiger tube and an electronic scaler. The counting geometry of the sample was kept constant by using a special, rigidly centered mounting device.

Methods for specific experiments

Wetting agents used with the labeled phosphate are given in Table 1. The solution was placed on the plants and allowed to remain for one week. Then each treated leaf was flooded with a Dreft solution, scrubbed with a soft brush, and rinsed thoroughly with distilled water. This cycle was repeated three times, after which the leaf was blotted dry with tissue. The treated leaf was then included with the analyzed material.

The effect of temperature on the absorption of P^{32} was studied by immediately placing treated plants into the tempera-

Table 1. Source, composition and classification of wetting agents used

Name	Source	Composition	Class
Dreft	Procter and Gamble Co.	Sodium lauryl sulfate	Anionic
Dodecyl alcohol	Distillation Products Co.	---	---
Triton B-1956	Rohm and Haas Co.	Modified phthalic glycerol alkyd resin in ethylene dichloride	Nonionic
Triton X-100	Rohm and Haas Co.	Alkylaryl polyether alcohol	Nonionic
Tween 20	Atlas Powder Co.	Condensation product of ethylene oxide with sorbitan monolaurate	Nonionic

ture chambers. After 24 hours the plants were removed from the chambers, the treated leaves washed, as described above, and placed with the tops for analysis. In some of the experiments the washings were collected quantitatively and then transferred to 50 ml. volumetric flasks. One ml. of the washings from each treatment was placed in a stainless steel counting cup and counted with the same equipment used to count briquets.

Since the absorption of P^{32} had a temperature coefficient of one (Table 15), no preliminary absorption period was used when studying the effects of temperature on the translocation of P^{32}. Treated plants were placed directly into the temperature chambers and allowed to remain for 24 hours. As they were removed the treated leaves and petioles were removed to prevent further translocation.

A 5 per cent sucrose solution was used to determine the effects of added sugar on the translocation of P^{32} in carbohydrate depleted soybeans. If the sugar was applied acropetally, one-half of the leaf was dipped into the solution. Sucrose was applied to the lower half of leaves by painting it on the surface with a brush. These treatments necessitated a slight change in the position of the active phosphate solution. When the sugar was applied below, the phosphorus was placed about one-fourth the way from the tip of the leaf. The P^{32} was placed three-fourths the distance from the tip

when the sugar was above it. The sugar was applied first and the P^{32} solution immediately afterward.

In the first experiments with the accumulation of P^{32} by excised beet leaves, all of the petiole except a one-half inch section was removed from each leaf. The leaf was then spread with phosphate solution and left in 400 cp. light from fluorescent bulbs with a day length of 18 hours. At the end of 48 hours each leaf was washed as described for soybean leaves treated with P^{32}, the surface dried and a count rate meter used to estimate the length of exposure required. The leaves were placed against Kodak X-ray film and wrapped in black paper, then frozen and held during the exposure period.

Beet leaves were wrapped in aluminum foil and left on the plant for 48 hours in order to deplete them of carbohydrates. The darkened leaves were paired with lighted leaves of similar size on the same plant and both excised as described earlier. All leaves were spread with P^{32} solution and the darkened leaves again covered with foil. The leaves were then placed in the light chamber and allowed to remain for 48 hours, after which time they were removed and radiographs made of them.

Pairs of leaves from the same plant were used for experiments with the effects of replacing air with nitrogen on the accumulation of radiophosphorus in the veins of beet leaves. Three pairs were selected and treated with P^{32} solution in

each experiment. The leaves were placed in vacuum desiccators, one of a pair in each desiccator. The desiccators were evacuated to a pressure of 25 mm. Hg with a Cenco Hyvac pump. One desiccator was slowly filled with nitrogen washed with pyrogallol, and the other with air. This operation was repeated three more times. Little oxygen remained in the desiccator repeatedly filled with nitrogen. After 48 hours in these desiccators in the dark, radioautographs were made of the leaves. When these experiments produced poor results, air was removed from one desiccator in another way. A desiccator was flushed with nitrogen. The bottom of the desiccator was quickly filled with fresh pyrogallol, and the treated leaves placed above the pyrogallol. The desiccator was flushed with nitrogen for one hour then sealed off and placed in the dark. Radioautographs were made of the leaves after they were washed. It should be stressed that with these and all radioautograph experiments the leaves were frozen at -20° F. as quickly as possible after being placed in contact with the film.

Treated leaves were allowed six hours for the absorption of P^{32} and washed in the standard manner. Another 24 hours was allowed for accumulation of the phosphorus, after which the organic and inorganic phosphorus in the leaves was separated and analyzed. The method used for separation was that of Klein (1952) which he had adopted from Juni *et al.* (1948). This method is somewhat arbitrary but is considered

to give adequate separation of the organic and inorganic phosphorus in plants.

Statistical Methods

The numerical data were analyzed with the use of the analysis of variance technique. The appropriate L.S.D.'s at the .05 and .01 per cent levels may be found in the tables.

RESULTS

Absorption Studies

Rate of absorption

The absorption of 2,4-D has been studied in some detail (Hauser, 1955), but little is known concerning the absorption of P^{32} labeled phosphate by leaves. The experiments reported here were designed to yield information concerning the tracers to be used in translocation and thereby serve as guides in planning the translocation work.

The first experiments with Na-2,4-D measured the rate of absorption. An application of 100 ppm. of the herbicide showed that at this concentration not enough of this compound was absorbed in 30 minutes to reduce significantly the new growth of soybeans (see Tables 2 and 3). The plants receiving the zero exposure were treated and washed immediately. Only a few seconds elapsed after treatment before the treated leaves were dipped into the first wash water. Plants exposed for longer periods were left on the greenhouse bench until washed. To wash them, the leaves were dipped into four solutions of Dreft, then four solutions of tap water. In the experiment using 100 ppm. of 2,4-D there were indications of injury from 2,4-D, but the differences were not statistically

Table 2. Rate of absorption of sodium 2,4-D

Treatment	Exposure interval, min.	Green wt. new growth, g.
100 ppm. 2,4-D + 0.1% B-1956	0	2.43
" " " " " "	10	2.39
" " " " " "	20	2.14
" " " " " "	30	1.67
0.1% B-1956	30	2.28

Table 3. Analysis of variance for the data of Table 2

Source	D.f.	S.s.	M.s.	"F"
Total	24	14.259		
Reps	4	7.446	1.861	
Treatments	4	1.258	.314	N.S.
Error	16	5.555	.347	

significant. A second experiment, identical to the first except that 600 ppm. of 2,4-D was used, showed the herbicide absorbed more rapidly (Tables 4 and 5). Enough was absorbed in 30 minutes to reduce significantly the new growth of the treated plants. The plants that were washed immediately showed that the washing technique removed all injurious amounts of 2,4-D from the surface of the treated leaves. In

Table 4. Rate of absorption of sodium 2,4-D

Treatment	Exposure interval, min.	Green wt. new growth, g.[a]
500 ppm. 2,4-D + 0.1% B-1956	0	3.99
" " " " " "	10	2.40
" " " " " "	20	2.60
" " " " " "	30	2.26
0.1% B-1956	30	3.48

[a]L.S.D.'s: Treatments - (.05) = 1.07
(.01) = 1.48

Table 5. Analysis of variance for the data of Table 4

Source	D.f.	S.s.	M.s.	"F"
Total	24	27.478		
Reps	4	5.909	1.477	
Treatments	4	11.291	2.258	3.51*
Error	16	10.278	.642	

both experiments the plants receiving only B-1956 exhibited no symptoms of injury from the wetting agent.

Table 6 and 7 show the results of an experiment designed to measure the effects of 2,4-D concentration and time of contact with leaves on absorption into, and translocation from

Table 6. Effects of concentration and length of exposure on the absorption and translocation of Na-2,4-D

Concentration	Wetting agent	Exposure, hr.	Fate of leaf at end of exposure	Wt. new growth, g.[a]
None	None	--	Washed	1.66
None	0.1% B-1956	4	"	1.44
None	"	4	Removed	1.52
500 ppm.	"	1	Washed	1.28
"	"	2	"	.91
"	"	4	"	.62
"	"	1	Removed	1.47
"	"	2	"	1.71
"	"	4	"	1.63
1000 ppm.	"	1	Washed	1.22
"	"	2	"	.62
"	"	4	"	.94
"	"	1	Removed	1.43
"	"	2	"	1.60
"	"	4	"	1.69
2000 ppm.	"	1	Washed	.64
"	"	2	"	.55
"	"	4	"	1.04
"	"	1	Removed	1.63
"	"	2	"	1.52
"	"	4	"	1.28

[a]L.S.D.'s: Treatments - (.05) = .42
(.01) = .55

Table 7. Analysis of variance for the data in Table 6

Source	D.f.	S.s.	M.s.	"F"
Total	104	22.383		
Reps	4	.564	.141	
Treatments	20	14.269	.839	7.56**
Error	80	7.550	.111	

these organs. The chemical was applied to one unifoliate leaf of each plant by dipping the distal two-thirds of the leaf into the indicated solutions. After an interval of one, two, or four hours the treated leaves were either washed to remove unabsorbed chemical or cut from the plant. The growth responses of the plants on which the treated leaves were washed showed the total penetration of 2,4-D into the leaves. When the leaves were removed at the end of the exposure period the plants received only the chemical which could be absorbed and translocated from the treated leaves in the experimental interval.

When the leaves were removed at the end of the exposure period only the plants treated with 2000 ppm. for four hours showed any reduction of growth, and this response was not statistically significant. In sharp contrast, enough 2,4-D was absorbed from the 2000 ppm. treatment in one hour to cause a highly significant growth reduction when the leaf was washed and left attached to the plant. Injurious amounts of 2,4-D were absorbed in two hours, even at a concentration of 500 ppm.

Two points may be stressed in the data of Table 6. Absorption increased with concentration, but heavy applications of 2,4-D tended to reduce translocation. Young (1953) has shown that herbicidal treatments which are rapidly injurious reduce the functioning of the phloem. In this experiment

2000 ppm. 2,4-D left on the leaves for four hours before washing caused significantly less injury than an exposure of two hours. The four hour exposure caused visible injury to the treated leaves while the new growth exhibited less injury as a result of reduced translocation.

This experiment and the two experiments previously discussed formed the basis of later work in which attempts were made to separate the processes of absorption and translocation. On the basis of these experiments a concentration of 750 ppm. and an exposure interval of two hours were used to give absorption of a toxic quantity of 2,4-D without measurable translocation from the treated leaf.

Distribution of labeled phosphate in soybeans

Before the results of the experiments with the absorption of phosphorus are considered it is of interest to look at the distribution of the applied P^{32} in the experimental plants. Ten microliters of the phosphate solution was applied with a micropipette and the unabsorbed material removed after two hours. The treated plants were allowed to grow for one week, then separated as indicated in Table 8. The results are the average of the P^{32} contained in 20 plants. After separation each portion was weighed, ground and diluted with untreated soybean material to make a 1.5 g. briquet. Only

Table 8. Distribution of P^{32} in soybeans when applied to one unifoliate leaf [a]

Plant part	Total counts per min.	Percentage in part, treated leaf included	Percentage in part, treated leaf excluded	Concentration, counts per min. per g.
Bud and young trifoliate	56,745	8.94	26.22	77,735
Mature trifoliate	71,798	11.31	33.17	58,851
Mature trifoliate petiole	3,569	.56	1.65	35,690
Stem above unifoliates	9,969	1.57	4.61	35,603
Treated unifoliate	418,194	65.90	--	589,026
Treated petiole	1,263	.20	--	12,630
Washings, treated unifoliate	3,370,915	--	--	--
Untreated unifoliate	6,540	1.03	3.02	9,212
Untreated petiole	308	.05	.14	3,080
Stem below unifoliates	20,349	3.21	9.41	16,150
Cotyledons	787	.12	.37	1,830
Roots	45,414	7.11	20.85	27,717

[a] 15.84% of total applied penetrated into plant
5.40% of total applied translocated from treated leaf

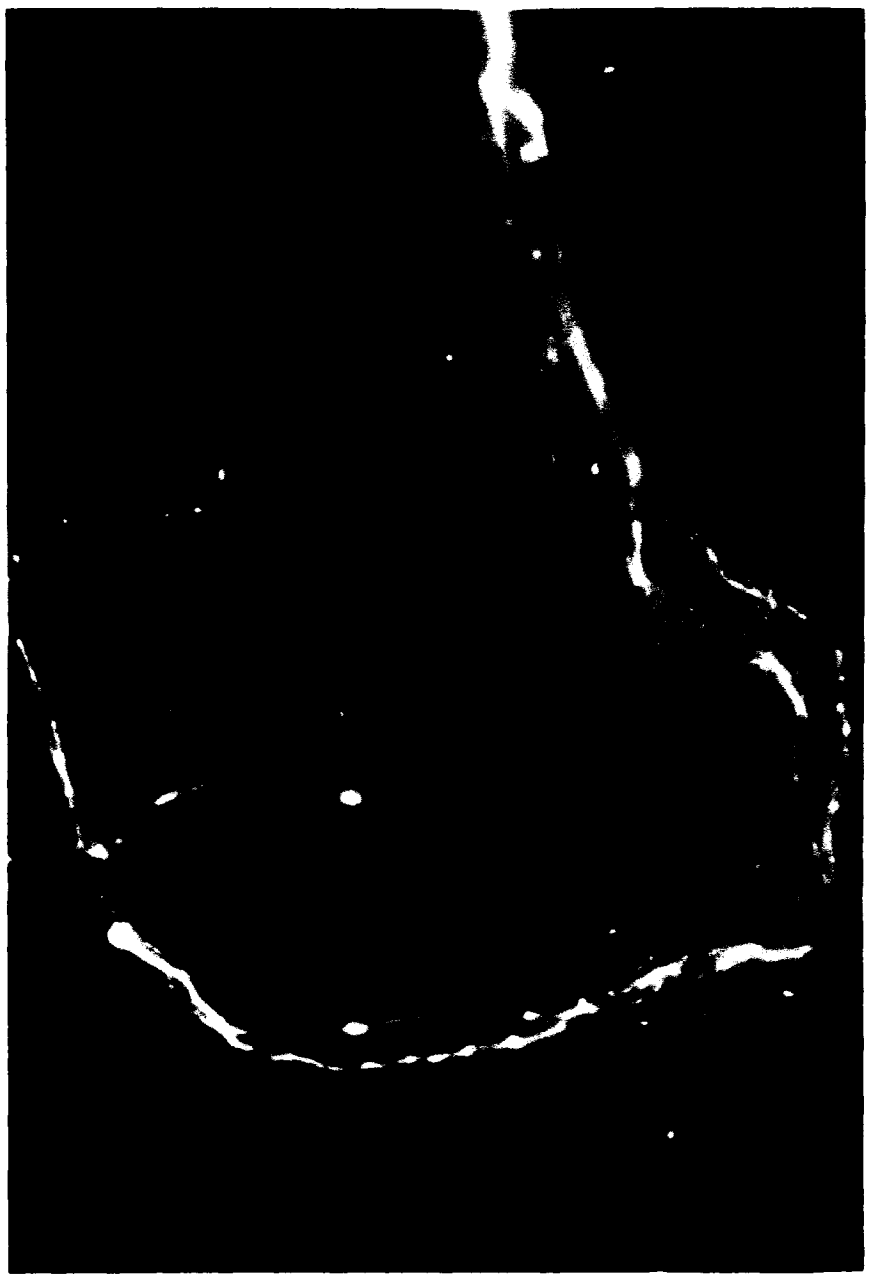
enough of the high activity material to give a counting rate of approximately 3000 counts per minute was included in the counting sample. Slightly less than one-sixth (15.8 per cent) of the applied P^{32} was absorbed by the plant leaves, and only about one-third (5.4 per cent) of this was translocated from the treated leaf. Over one-half of the translocated P^{32} was in the parts which had developed since treatment, the younger trifoliates and the bud. Of the remainder, one-fifth was in the roots, while other parts contained smaller quantities. The untreated unifoliate and the cotyledons contained only a small proportion of the P^{32} moved into the plant. The radioautographs in Figures 1 and 2 illustrate the distribution of P^{32} in these soybean plants. The treated leaf was removed at the point indicated by the arrow. The large quantity of P^{32} contained in it would have blurred much of the picture if it had been left attached.

Effects of wetting agents

Several workers have reported that the addition of surface active agents to 2,4-D sprays may greatly increase the rate and amount of the herbicide absorbed (Hitchcock and Zimmerman, 1948; Staniforth and Loomis, 1949; Hauser, 1955). Swanson and Whitney (1953) found that the one wetting agent they studied, "Tween 80", reduced the absorption of labeled

Figure 1. Distribution of P^{32} in the top of a treated soybean plant. P^{32} was applied to one unifoliate leaf, removed before making the exposure, which was attached at the point indicated by the arrow. Note the concentration of P^{32} in the bud and growing leaves and its near absence from the untreated unifoliate and from the older trifoliate leaf.

Figure 2. Distribution of P^{32} in the roots of a treated soybean plant. Root tips and primordia show concentration of the radioactive phosphorus.



phosphate. They postulated that a wetting agent-phosphate complex was formed.

A number of experiments were performed to study the effects of a variety of wetting agents on absorption of P^{32}. (See Table 1 for more complete information concerning the wetting agents used.) In the first experiments the total volume applied was 10 µl containing 0.1 per cent of the wetting agent (Tables 9 and 10). The results were variable but none of the wetting agents significantly affected the amount of P^{32} absorbed in one week of exposure. In one of the experiments, dodecyl alcohol was included in an effort to find a less reactive molecule that might behave as a surfactant. The emulsion formed by mixing this with the treatment was not absorbed in a greater quantity than the phosphate solution alone. In these experiments only one-fifth as much active phosphate solution was used as was applied by Swanson and Whitney (1953), and the volume was so small that even with a wetting agent not much of the leaf was covered.

The total volume of solution was doubled in the next experiments, thereby doubling the quantity of wetting agent present without changing the quantity of P^{32}. This change appeared to bring out differences in the effects of wetting agents on the absorption of labeled phosphate (Tables 11 and 12). Two of the wetting agents, B-1956 and Dreft, greatly decreased the absorption of foliar applications of phosphate,

Table 9. Effects of 0.1 per cent wetting agent on the absorption of P^{32} when applied in 10 µl of solution

Wetting agent	Counts per minute	
	Expt. 1	Expt. 2
None	1,326	2,067
B-1956	1,901	2,018
Tween-20	2,399	2,355
Dreft	1,783	1,687
Dodecyl alcohol	--	1,956

Table 10. Analyses of variance for the data of Table 9

Source	D.f.	S.s.	M.s.	"F"
		Experiment 1		
Total	23	132,580,663		
Reps	5	19,353,534	3,910,707	
Treatments	3	38,832,625	12,944,209	2.61 N.S.
Error	15	74,394,504	4,959,634	
		Experiment 2		
Total	29	20,447,230		
Reps	5	2,321,433		
Treatments	4	1,222,881	305,720	-- N.S.
Error	20	16,902,915	845,146	

Table 11. Effects of 0.1 per cent wetting agent on the absorption of P^{32} when applied in 20 µl of solution

Wetting agent	Counts per minute Expt. 1[a]	Expt. 2[a]
None	7,968	6,421
B-1956	1,112	984
Tween-20	10,073	8,144
Dreft	2,592	2,028
Dodecyl alcohol	4,727	4,870
X-100	8,849	7,489

[a]L.S.D.'s: Treatments - (.05) Expt. 1 = 3,961
Expt. 2 = 3,321
(.01) Expt. 1 = 5,403
Expt. 2 = 4,529

Table 12. Analyses of variance for the data in Table 11

Source	D.f.	S.s.	M.s.	"F"
		Experiment 1		
Total	29	401,724,275		
Reps	4	10,947,250	2,736,813	
Treatments	5	210,326,086	42,065,217	3.00*
Error	20	180,450,939	9,022,547	
		Experiment 2		
Total	29	414,944,787		
Reps	4	22,741,209	5,685,302	
Treatments	5	215,372,036	43,074,407	4.71*
Error	20	176,831,542	8,841,577	

possibly because of the formation of complexes with P^{32}. Two more wetting agents, Tween 20 and X-100, tended to increase the absorption of $P^{32}O_4^{\equiv}$, although this increase was small and not statistically significant. The wetting agents were observed to cause the phosphate solution to spread over the leaves as it was applied with a pipette. Although more leaf area was exposed to the solution, this wetting action had little effect on absorption of the phosphate. These results with an inorganic phosphate ion are in sharp contrast to those obtained with the organic molecules of 2,4-D.

Effects of temperature

It has been shown that temperature may affect the rate of absorption of foliar applications of 2,4-D (Bryan et al., 1950; Hauser, 1955). The data in Tables 13 and 14 are in agreement with the conclusions of these workers. The plants were treated and placed immediately in 15 or 30° temperature chambers for two hours. The treated leaves were washed as the plants were removed from the chambers. The effect of temperature shows as a significant interaction of 2,4-D and temperature. Inspection of the data reveals that this interaction was caused by the reduction of new growth of treated plants at the higher temperature. The temperature coefficient for the absorption of 2,4-D was approximately 2.

Table 13. The effect of temperature on the absorption of 750 ppm. Na-2,4-D (0.1 per cent B-1956 added)

2,4-D treatment	Temperature, degrees C.	Green wt. new growth, g.[a]
None	15	3.86
2,4-D	15	1.09
None	30	4.35
2,4-D	30	.60

[a]L.S.D.'s: Treatments - (.05) = .72
(.01) = 1.09

Table 14. Analysis of variance for the data in Table 13

Source	D.f.	S.s.	M.s.	"F"
Total	23	68.748		
Reps	5	1.518	.306	
Treatments	3	62.472	20.824	61.2**
2,4-D	1	60.420	60.420	177.6**
temperature	1	.041	.041	N.S.
2,4-D x temperature	1	2.010	2.010	5.93*
Error	15	4.758	.340	

Other experiments indicated that increasing the temperature from 15 to 30° C. had little effect on the absorption of P^{32} (Tables 15 and 16). Treated plants were placed in chambers at the desired temperature and left for 24 hours. As the plants were removed each treated leaf was flushed with a

Table 15. Effect of temperature on the rate of absorption of P^{32}

Temperature, degrees C.	Counts per minute			
	Plants		Washings	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2[a]
15	6,023	2,905	2,892	2,357
30	5,856	3,275	2,857	1,525

[a]L.S.D.'s: Washings of Expt. 2 - (.05) = 572
(.01) = 847

Table 16. Analyses of variance for the data in Table 15

Source	D.f.	S.s.	M.s.	"F"
		Plants		
		Experiment 1		
Total	15	27,600,074		
Reps	7	8,201,682	1,171,669	
Temperatures	1	146,689	146,689	N.S.
Error	7	19,251,703	2,570,243	
		Experiment 2		
Total	15	9,685,864		
Reps	7	1,889,906	269,987	
Temperatures	1	545,751	545,751	
Error	1	7,250,181	1,035,754	

Table 16. (Continued)

Source	D.f.	S.s.	M.s.	"F"
		Washings		
		Experiment 1		
Total	15	722,306		
Reps	7	376,386	53,769	
Temperatures	1	3,969	3,969	N.S.
Error	7	231,951	33,136	
		Experiment 2		
Total	15	7,957,471		
Reps	7	3,555,216	507,888	
Temperatures	1	2,764,738	2,764,738	11.8*
Error	7	1,637,517	233,931	

Dreft solution, scrubbed with a brush and rinsed. This cycle was repeated three times and the washings collected. The washings from the treated leaves, as well as the plants, were analyzed. The counts from one set of washings had significantly less P^{32} at the 30° temperature, suggesting a greater absorption at the higher temperature. This difference may have been due to losses in the washing process. It was difficult to scrub the leaves with a brush and get quantitative transfer of the P^{32} on them. There are no indications in the remaining data that a greater quantity of phosphate was absorbed at 30° C. These results, also, point up the differences in the behavior of an inorganic ion and an organic

molecule.

When 2,4-D is absorbed by plant roots it has been found to move in the xylem (Ferri, 1945; Weaver and DeRose, 1946; Mitchell and Brown, 1946). These workers did not investigate the effects of temperature on the absorption of 2,4-D by plant roots. An experiment was performed to investigate this factor. Soybean plants were grown in nutrient culture, two plants to each jar. When the unifoliate leaves were fully expanded but the trifoliates were still rolled in the bud, the jars containing the experimental plants were placed in two water baths. One bath was maintained at 13° C. and the other at 23° C. After equilibration the indicated rates of Na-2,4-D were placed in the nutrient cultures at the two temperatures. The plant tops were maintained at the greenhouse temperature while the roots were subjected to the 2,4-D and temperature treatments for 24 hours. After this time had elapsed the roots were washed and placed in fresh nutrient solution at 23° C. Fourteen days later the weight of new growth on each plant was recorded. The plants exposed to 2,4-D were badly injured, but this injury was independent of temperature (Tables 17 and 18). The Q_{10} for absorption of the 45 ppm. concentration was 1.08, and for the 135 ppm. rate the Q_{10} was .99. This experiment showed a much greater absorption and translocation of 2,4-D by roots compared with 2,4-D applied to leaves.

Table 17. Effect of temperature on the absorption of Na-2,4-D by soybean roots (exposed for 24 hours)

2,4-D, ppm.	Root temperature, degrees C.	Green wt. new growth, g.[a,b]
0		1.16
45	13	0.39
135		0.10
0		1.40
45	23	0.42
135		0.14

[a] L.S.D.'s: Treatments - (.05) = .24
(.01) = .35

[b] Q_{10} at 45 ppm. = 1.08
Q_{10} at 135 ppm. = .99

Table 18. Analysis of variance for the data in Table 17

Source	D.f.	S.s.	M.s.	"F"
Total	35	2.57323		
Reps	2	.08843	.04422	
Treatments	5	2.23325		
temperature	1	.02507	.02507	1.40 N.S.
rates	2	2.18567	1.09284	61.2**
temperature x rates	2	.02251	.01125	N.S.
Plants within jars	18	.07270	.00404	N.S.
Error	10	.17885	.01786	

Effects of the carbohydrate supply of leaves

Hauser (1955) has reported that the level of carbohydrates in soybean leaves did not affect the rate of 2,4-D absorption. The experiments reported here were designed to measure the effects of added sucrose on the absorption of 2,4-D by soybean leaves depleted of carbohydrates by placing them in the dark for 24 hours. Soybean plants were treated with a solution containing 750 ppm. of 2,4-D and 5 per cent sucrose. After treatment the plants were returned to the dark for another 24 hours. As the plants were brought into the light again, the treated leaves were washed to remove any unabsorbed 2,4-D. The sucrose which was included in the treatment did not increase the absorption of 2,4-D (Tables 19 and 20).

In contrast to the results of Hauser (1955) with 2,4-D, placing the test plants in the dark for 24 hours prior to treatment decreased the rate of absorption of labeled phosphate (Tables 21 and 22). Plants in the light before treatment and the dark after treatment absorbed more phosphate than any of the plants in the dark before treatment. Whether the plants were in the light or dark after treatment had little effect on the quantity of phosphate absorbed during the 24-hour exposure. If carbohydrates alone were responsible, the leaves in the light after treatment should have

Table 19. Effect of sucrose on absorption of 2,4-D

Treatment	Green wt. new growth, g. Expt. 1[a]	Expt. 2[a]
.1% B-1956	1.09	1.25
.1% B-1956 + 5% sucrose	.92	1.31
.1% B-1956 + 750 ppm. 2,4-D	.63	.77
.1% B-1956 + 750 ppm. 2,4-D + 5% sucrose	.35	.88

[a]L.S.D.'s: Treatments - (.05) Expt. 1 = .32
Expt. 2 = .47
(.01) Expt. 1 = .45
Expt. 2 = .66

Table 20. Analyses of variance for the data in Table 19

Source	D.f.		S.s.	M.s.	"F"
			Experiment 1		
Total	19		2.397		
Reps	4		.190	.048	
Treatments	3		1.585	.528	9.8**
2,4-D		1	1.311	1.311	24.3**
sucrose		1	.207	.207	3.83 N.S.
2,4-D x sucrose		1	.067	.067	1.24 N.S.
Error	12		.622	.054	
			Experiment 2		
Total	19		3.486		
Reps	4		.324	.081	
Treatments	3		1.761	.587	5.02*
2,4-D		1	1.557	1.557	13.3**
sucrose		1	.173	.173	1.48 N.S.
2,4-D x sucrose		1	.031	.031	N.S.
Error	12		1.401	.117	

Table 21. Effect of readily available carbohydrates on the absorption of P^{32}

24 hrs. before treatment	24 hrs. after treatment	Counts per minute Expt. 1[a]	Expt. 2[a]
Light	Light	5,920	3,814
Light	Dark	7,099	4,245
Dark	Light	4,858	2,206
Dark	Dark	4,554	2,706

[a]L.S.D.'s: Treatments - (.05) Expt. 1 = 1,686
Expt. 2 = 1,070
(.01) Expt. 1 = 2,224
Expt. 2 = 1,474

Table 22. Analyses of variance for the data in Table 21

Source	D.f.	S.s.	M.s.	"F"
		Experiment 1		
Total	23	57,914,089		
Reps	5	5,621,970	1,124,394	
Treatments	3	23,952,849	7,984,283	4.23*
before	1	19,506,657	19,506,657	10.32**
after	1	1,148,000	1,148,000	N.S.
before x after	1	3,298,192	3,298,192	1.75 N.S.
Error	15	28,339,270	1,889,284	
		Experiment 2		
Total	23	27,714,948		
Reps	5	316,262	63,252	
Treatments	3	16,067,038	5,355,679	7.09**
before	1	14,709,438	14,709,438	19.5**
after	1	1,346,634	1,346,634	1.78 N.S.
before x after	1	10,966	10,966	N.S.
Error	15	11,331,648	755,443	

absorbed more P^{32} than the ones remaining in the dark. The relationship is probably a complex one, possibly involving the effect of light on the electrical charge of the leaf.

Translocation Studies

Effects of temperature

Previous experiments had shown that absorption and translocation can be separated on differences in rate, but did not determine the time necessary to obtain transport of toxic amounts of 2,4-D from treated leaves. Since temperature was assumed to influence the rate of translocation, it was important to know the length of exposure needed at known temperatures to obtain transport of enough 2,4-D to reduce plant growth. The first experiment with translocation which contained temperature as a variable involved three lengths of exposure (one, five and 25 hours). One unifoliate leaf of each plant was treated by dipping, and all plants were held at a uniform temperature for two hours to allow absorption of the applied 2,4-D. The treated leaves were then washed and the plants placed in temperature chambers at 5, 15 or 30° C. After the desired exposure time had elapsed, the treated leaves were removed and the plants returned to a greenhouse bench. After 10 days in the greenhouse, the new growth was

removed and the green weight taken as a measure of the 2,4-D translocated.

A translocation time of one or five hours caused no significant reductions in growth at any temperature (Tables 23 and 24). When the treated leaves were left on the plants for 25 hours there were significant differences in growth. More 2,4-D was translocated at 30° C. than at 15°. There was no reduction in growth at 5° C., even when the leaves were exposed for 25 hours. The temperature coefficient between 15 and 30° C. for the translocation of 2,4-D was 1.63 in this experiment. This temperature coefficient and the others given for later experiments with 2,4-D were calculated from the percentage reduction in growth compared with check plants held at the same temperature.

In a second experiment two time intervals intermediate between those used before were selected, with temperatures of 5, 15 and 25° C. (Table 25). During four hours there was not enough 2,4-D translocated at any temperature to cause a significant reduction in growth (Tables 25 and 26). Extremely toxic amounts of 2,4-D were translocated at 15 and 25° C. during a 16 hour exposure. At the lowest temperature the reduction was not significant even after 16 hours. The percentage reduction of new growth increased with temperature. The Q_{10} for translocation between 5 and 15° C. was 1.67. Between 15 and 25° C. the Q_{10} was 1.64.

Table 23. Effects of temperature and time of exposure on translocation of 2,4-D (all plants treated with 0.1% B-1956)

2,4-D, ppm.	Length of exposure, hr.	Temperature, degrees C.	Wt. of new growth, g.[a,b]
750	1	5	1.29
"	5	5	1.12
"	25	5	1.07
"	1	15	.94
"	5	15	.89
"	25	15	.87
"	1	30	1.26
"	5	30	.94
"	25	30	.68
0	25	5	1.06
"	25	15	1.04
"	25	30	1.03

[a] L.S.D.'s: Treatments - (.05) = .29
(.01) = .39

[b] Q_{10} between 15 and 30° for the 25 hr. exposure = 1.63

Table 24. Analysis of variance for the data in Table 23

Source	D.f.	S.s.	M.s.	"F"
Total	59	4.200		
Reps	4	.322	.081	
Treatments	11	1.572	.143	2.75*
Error	44	2.306	.052	

Table 25. Effect of two times of exposure, two rates of 2,4-D and three temperatures on translocation of 2,4-D (0.1% B-1956 applied to all plants)

Exposure, hrs.	2,4-D, ppm.	Temperature, degrees C.	Wt. of new growth, g.[a,b]
4	0	5	3.00
16	0	5	2.97
4	750	5	2.97
16	750	5	2.67
4	0	15	2.81
16	0	15	2.87
4	750	15	2.84
16	750	15	2.29
4	0	25	3.06
16	0	25	2.93
4	750	25	2.68
16	750	25	2.04

[a] L.S.D.'s: Treatments - (.05) = .53
(.01) = .71

[b] Q_{10} between 15 and 25° C. for 16 hr. exposure = 1.64
Q_{10} between 5 and 15° C. for 16 hr. exposure = 1.67

Table 26. Analysis of variance for the data in Table 25

Source	D.f.	S.s.	M.s.	"F"
Total	119	56.023		
Reps	9	2.782	.309	
Treatments	11	17.507	1.945	5.39**
time	1	2.015	2.015	5.58*
temp.	2	1.400	.700	1.94 N.S.
rate of 2,4-D	1	3.488	3.488	9.67**
time x temp.	2	2.940	1.470	4.07*
time x rate	1	2.341	2.341	6.21*
temp. x rate	2	.944	.472	1.31 N.S.
time x temp. x rate	2	4.379	2.190	6.07**
Error	99	35.726	.3609	

In a third experiment a uniform exposure of 24 hours was used because this interval appeared desirable from the previous experiments. The experiment was set up in a factorial design and 10 replicates were used to measure more accurately the effects of temperature on the rate of translocation of 2,4-D from soybean leaves. The weight of the new growth of plants held at 15° C. during the translocation period was considerably greater than the growth on plants held at 30° C. (Tables 27 and 28). In both instances enough 2,4-D was translocated from the treated leaves to injure the plants. In this experiment the temperature coefficient for the translocation of 2,4-D was 1.79. The Q_{10}'s from this experiment and the two above are in the range typical of chemical reactions. They indicate that the rate of translocation of 2,4-D is limited by a chemical reaction.

Several experiments on the effects of temperature on the transport of labeled phosphate from leaves were performed. The labeled phosphate ion in weakly acid solution was applied to the center of one unifoliate leaf of each plant. Approximately 1 μc of P^{32} in 10 μl of solution was used. In a single experiment the same solution was used to treat all plants, therefore, the rate was uniform for a given experiment but varied somewhat between experiments. The plants were placed in the temperature chambers without an absorption interval, for the absorption of P^{32} was found to have a

Table 27. Effect of temperature on the translocation of 2,4-D (all plants received 0.1% B-1956)

2,4-D, ppm.	Temperature, degrees C.	Wt. new growth, g.[a,b]
0	15	1.89
750	15	1.62
0	30	1.86
750	30	1.22

[a] L.S.D.'s: Treatments - (.05) = .23
(.01) = .31

[b] Q_{10} = 1.79

Table 28. Analysis of variance for the data in Table 27

Source	D.f.	S.s.	M.s.	"F"
Total	39	5.5780		
Reps	9	1.0005	0.1112	
Treatments	3	2.8683		
2,4-D	1	2.0977	2.0977	33.14**
temp.	1	.4537	.4537	7.17*
2,4-D x temp.	1	.3169	.3169	5.00*
Error	27	1.7092	.0633	

temperature coefficient of one (Table 15). After 24 hours the plants were taken from the chambers and the treated leaves removed from one-half of the plants held at each temperature. All plants were returned to the greenhouse for a week. At the end of this time the treated leaves were removed from the remainder of the plants, and all plants were harvested and dried in preparation for assay. The briquet method was used to assay the P^{32} contained in the plants.

Almost no P^{32} was translocated from the treated leaves when the plants were held at 5° C. (Tables 29 and 30). The plants that were exposed at 5° C. and the leaves left attached after removal from the temperature chamber contained much more P^{32}. At 30° C. more P^{32} was translocated than at 5° C. The Q_{10} for the translocation of P^{32} in this experiment was 2.17.

The rates of translocation of P^{32} at 15 and 30° C. were compared in two additional experiments similar to the one previously described. In both experiments less P^{32} was translocated at the lower temperature (Tables 31, 32, 33 and 34). The temperature coefficient for one experiment was 2.37 and for the other 2.05. The effects of temperature on the translocation of P^{32} and 2,4-D were similar. It appears that the movement of both materials is controlled by a biochemical mechanism. These results suggest that the 2,4-D and P^{32}

Table 29. Effects of temperature on translocation of P^{32} (all plants treated with 1 μc of labeled phosphate in 10 μl of solution)

Temperature, degrees C.	Fate of treated leaf when removed from temperature chamber	Counts per min.[a,b]
5	Not removed	6,113
5	Removed	365
30	Not removed	10,088
30	Removed	2,531

[a] L.S.D.'s: Treatments - (.05) = 1,251
(.01) = 1,730

[b] Q_{10} = 2.17

Table 30. Analysis of variance for the data in Table 29

Source	D.f.	S.s.	M.s.	"F"
Total	23	53,061,907		
Reps	5	5,012,197		
Treatments	3	37,709,254	12,569,751	18.2**
temperature	1	6,904,913	6,904,913	10.0**
removal of leaf	1	29,426,042	29,426,042	42.7**
temp. x removal	1	1,378,299	1,378,299	2.0 N.S.
Error	15	10,341,499	689,433	

Table 31. Effects of temperature on the translocation of P^{32} during a 24 hour exposure (all plants treated with 1 μc of labeled phosphate in 10 μl of solution)

Temperature, degrees C.	Fate of treated leaf when removed from temperature chamber	Counts per min.[a,b]
15	Not removed	2,878
15	Removed	450
30	Not removed	4,019
30	Removed	1,644

[a] L.S.D.'s: Treatments - (.05) = 1,004
(.01) = 1,586

[b] Q_{10} = 2.37

Table 32. Analysis of variance for the data in Table 31

Source	D.f.	S.s.	M.s.	"F"
Total	15	39,888,754		
Reps	3	6,816,882	2,272,294	
Treatments	3	28,520,435	9,506,812	18.8**
temperature	1	5,446,389	5,446,389	10.4*
removal of leaf	1	23,071,211	23,071,211	45.6**
temp. x removal	1	2,835	2,835	N.S.
Error	9	4,554,272	506,030	

Table 33. Effects of temperature on translocation of P^{32} in a 24 hour exposure (all plants treated with 1 µc of labeled phosphate in 10 µl of solution)

Temperature, degrees C.	Fate of leaf after exposure	Counts per min.[a,b]
15	Not removed	2,133
15	Removed	353
30	Not removed	2,361
30	Removed	1,037

[a] L.S.D.'s: Treatments - (.05) = 507
(.01) = 701

[b] Q_{10} = 2.05

Table 34. Analysis of variance for the data in Table 33

Source	D.f.	S.s.	M.s.	"F"
Total	23	20,561,669		
Reps	5	2,005,738	401,148	
Treatments	3	16,005,126	5,335,042	31.4**
removal of leaf	1	14,444,465	14,444,465	84.9**
temperature	1	1,248,984	1,248,984	7.34*
removal x temp.	1	311,677	311,677	1.83 N.S.
Error	15	2,550,805	170,054	

moved from the leaves largely, if not entirely, in the phloem.

Effects of the carbohydrate supply to leaves

A supply of carbohydrates in the leaf has been shown to be necessary for the translocation of foliar applications of 2,4-D in several species (Mitchell and Brown, 1946; Weaver and DeRose, 1946; Rohrbaugh and Rice, 1949; Weintraub and Brown, 1950). No work was done with soybeans, for most of these authors confined their experiments to kidney beans. The experiments reported here using soybeans as test plants show that the supply of carbohydrates in the leaves affects the translocation of 2,4-D in this plant also. Plants which had been in the dark for 24 hours were treated by dipping two-thirds of one unifoliate leaf in the solutions indicated in Table 35. One treatment consisted of 5 per cent sucrose with 0.1 per cent B-1956; another contained Na-2,4-D, sucrose and B-1956; while the third consisted of 2,4-D and the wetting agent. All plants were returned to the dark for 24 hours. As the plants were brought into the light again the treated leaves on one half of each treatment were removed, while they were left attached on the other half. The new growth of the latter plants served as checks on the amount of 2,4-D present in the leaves. The new growth of the plants with the leaves removed measured the amount of 2,4-D translocated during the

experimental interval.

There was no reduction in the trifoliate growth of plants treated with 2,4-D alone, and with treated leaves removed before the plants were returned to the light, while plants handled in the same manner but receiving 5 per cent sucrose in the treatment showed a marked reduction in new growth (Tables 35 and 36). The added sucrose without 2,4-D had no significant effect on the growth of the plants.

From the work already reported in this thesis it might be anticipated that P^{32} applied to carbohydrate depleted soybean leaves would behave in a manner similar to 2,4-D. Plants depleted of carbohydrates were divided into three lots, of which one was treated with sucrose and the other two lots were left untreated. The sugar was applied to one leaf on each plant by dipping one half of it into a 5 per cent solution. Following the sucrose treatment all plants were treated with labeled phosphate half way between the basipetal extremity of the sucrose and the base of the leaf. The plants treated with sucrose and one set of the plants not receiving sucrose were returned to the dark, while the other set was allowed to remain in full light. After 24 hours all treated leaves and petioles were removed and the plants in the dark returned to the light. One week later the plants were harvested and dried. This material was assayed for P^{32} by the briquet method previously described.

Table 35. Effects of sucrose on the translocation of 2,4-D from carbohydrate depleted leaves of soybeans (exposed to treatment for 24 hours in the dark; 0.1% B-1956 in all treatments)

Sucrose, per cent	2,4-D, ppm.	Fate of treated leaf as plant was removed from dark	Wt. new growth, g.[a]
5	0	Left attached	1.11
5	0	Removed	.99
5	750	Left attached	.67
5	750	Removed	.79
0	750	Left attached	.59
0	750	Removed	1.16

[a]L.S.D.'s: Treatments - (.05) = .24
(.01) = .33

Table 36. Analysis of variance for the data in Table 35

Source	D.f.	S.s.	M.s.	"F"
Total	29	2.328		
Reps	4	.280	.070	
Treatments	5	1.330	.266	7.71**
Error	20	.718	.035	

Sucrose supplied above the phosphate greatly increased the P^{32} translocated from carbohydrate depleted leaves (see Tables 37 and 38). The plants in the light showed considerably more transport of P^{32} than any plants in the dark. This was probably caused by the larger amount of carbohydrate available for translocation in the leaves carrying on photosynthesis. These results indicate another similarity between the translocation of foliar applications of 2,4-D and P^{32}.

The experiment above led to a question of the effects of sucrose applied below the labeled phosphate on transport from carbohydrate depleted leaves. In two experiments sucrose was applied to the basal half of the treated leaf, and phosphate was applied half way between the distal extremity of the sucrose and the leaf tip. The sucrose was applied by painting a 5 per cent solution on both surfaces of the treated leaves with a small, soft brush. Other procedures were the same as for the previous experiment. Plants with and without sucrose were placed in the light and in the dark for a 24 hour period after treatment. When the plants were in the dark the application of sucrose below the phosphate did not increase the quantity of P^{32} translocated (Tables 39, 40, 41 and 42). A probable explanation of this response is that the sucrose was transported downward from the point of application and did not reach the tissues containing P^{32}. Therefore, the sucrose was not available to aid in the trans-

Table 37. Effects of sucrose on the translocation of P^{32} from carbohydrate depleted leaves of soybeans (all plants received 1 µc P^{32} in 10 µl of solution; all treated leaves removed after a 24 hour exposure)

Treatment during exposure	Sucrose	Counts per min.[a]
Dark	0	749
Dark	5%	4,791
Light	0	15,701

[a]L.S.D.'s: Treatments - (.05) = 3,340
(.01) = 4,683

Table 38. Analysis of variance for the data in Table 37

Source	D.f.	S.s.	M.s.	"F"
Total	20	1,090,891,000		
Reps	6	156,468,000		
Treatments	2	835,720,000	417,860,000	50.8**
Error	12	98,703,000	8,225,000	

Table 39. Effects of sucrose applied below the phosphate on the translocation of P^{32} from carbohydrate depleted soybean leaves

24 hrs. after treatment	Sucrose	Counts per min.[a]
Light	0	4,487
Dark	0	3,241
Light	5%	19,771
Dark	5%	1,429

[a]L.S.D.'s: Treatments - (.05) = 1,206
(.01) = 1,668

Table 40. Analysis of variance for the data in Table 39

Source	D.f.	S.s.	M.s.	"F"
Total	23	1,311,180,000		
Reps	5	8,843,000	1,769,000	
Treatments	3	1,287,927,000	429,309,000	447**
sucrose	1	274,280,000	274,280,000	285**
light	1	572,600,000	572,600,000	596**
sucrose x light	1	432,204,000	432,204,000	450**
Error	15	14,411,000	961,000	

Table 41. Effects of sucrose applied below the phosphate on translocation of P^{32} from carbohydrate depleted soybean leaves

24 hrs. after treatment	Sucrose	Counts per min.[a]
Light	0	1,573
Dark	0	565
Light	5%	1,452
Dark	5%	606

[a]L.S.D.'s: Treatments - (.05) = 343
(.01) = 474

Table 42. Analysis of variance for the data in Table 41

Source	D.f.	S.s.	M.s.	"F"
Total	23	6,690,003		
Reps	5	178,144	35,629	
Treatments	3	5,245,174	1,748,391	22.5**
sucrose	1	7,632	7,632	N.S.
light	1	5,202,428	5,202,428	66.9**
sucrose x light	1	35,114	35,428	N.S.
Error	15	1,166,685	77,779	

port of phosphorus. No experiments of this type were performed with 2,4-D because not enough 2,4-D and sucrose could be applied to separated portions of one soybean leaf.

When plants low in carbohydrates were treated with 2,4-D and allowed to remain in the dark for 24 hours before being returned to the light with the treated leaves attached, 2,4-D seemed to be translocated out of the treated leaves in a normal manner (Table 35). These results indicate that absorbed 2,4-D is held in the leaves and is ready for transport when carbohydrates become available. In order to investigate the effects on translocation of sucrose supplied after the 2,4-D, applications of this sugar were made six and 12 hours after the application of 2,4-D. The sucrose was applied by dipping each treated leaf into a fresh beaker of 5 per cent solution. Each leaf not receiving sucrose was dipped in a fresh beaker of water. In this way 2,4-D was washed uniformly from the leaves, with and without supplying sucrose to them. This treatment eliminated, also, any effects that wetting the leaf might have on the amount of 2,4-D absorbed.

In the first experiment one half the plants received 2,4-D and one half did not (Table 43). In the second experiment all plants received 2,4-D (Table 45). In both experiments the time that sucrose was applied had no effect (Tables 43, 44, 45 and 46). In the second experiment the application of sucrose at either time caused a significant reduction in

Table 43. Effect of time of application of sucrose to carbohydrate depleted leaves on the translocation of 2,4-D (0.1% B-1956 and 5% sucrose applied to all plants)

2,4-D treatment	Delay in sucrose application, hr.	Green wt. new growth, g.[a]
None	6	1.05
None	12	.99
750 ppm.	6	.78
750 ppm.	12	.76

[a]L.S.D.'s; Treatments - (.05) = .16
(.01) = .23

Table 44. Analysis of variance for the data in Table 43

Source	D.f.	S.s.	M.s.	"F"
Total	19	.6680		
Reps	4	.1823	.0456	
Treatments	3	.3161	.1054	7.48**
time	1	.0066	.0066	N.S.
2,4-D	1	.3076	.3076	21.8**
time x 2,4-D	1	.0029	.0029	N.S.
Error	12	.1696	.0141	

Table 45. Effects of time of application of sucrose to carbohydrate depleted soybean leaves on the translocation of 2,4-D (all plants treated with a solution containing 750 ppm. of 2,4-D and 0.1% B-1956)

Sucrose treatment	Delay in sucrose application, hr.	Green wt. new growth, g.[a]
None	6	3.53
5%	6	2.38
None	12	3.56
5%	12	2.64

[a]L.S.D.'s: Treatments - (.05) = .64
(.01) = .87

Table 46. Analysis of variance for the data in Table 45

Source	D.f.	S.s.	M.s.	"F"
Total	31	18.406		
Reps	7	1.620	.231	
Treatments	3	8.629	2.876	7.61**
time	1	.254	.254	N.S.
sucrose	1	8.211	8.211	21.7**
time x sucrose	1	.164	.164	N.S.
Error	21	8.157	.378	

new growth compared to plants treated with 2,4-D but not receiving sucrose (Table 45). The second experiment also points up the increased translocation caused by sucrose supplied to carbohydrate depleted leaves.

Effects of the application of 2,4-D in oil

Rice and Rohrbaugh (1953) reported that the application of 2,4-D in kerosene caused the herbicide to move out of leaves depleted of carbohydrates. The effects on translocation from soybean leaves of using oil as a carrier for 2,4-D were investigated. The oil used was highly refined and non-phytotoxic, but very similar to kerosene in other respects. The propyleneglycolbutylether ester of 2,4-D was applied in this oil and in a water emulsion, to soybean leaves depleted of carbohydrates. The treatments were applied with a pipette in a total volume of 50 µl. Treatments were placed in aluminum rings sealed to the leaves with agar as described by Swanson and Whitney (1953). These rings prevented the oil and 2,4-D from spreading over the surface of the plant. Any plants with rings that leaked were rejected and new ones treated. Leaks were easily detected, for the oil could be seen to spread rapidly over the leaf. After treatment the plants were returned to the dark for 24 hours. At the end of this period all treated leaves and petioles were removed and

the plants returned to the light.

The new growth of plants treated with the ester of 2,4-D in oil was severely curtailed, while little injury was apparent on plants treated with the same ester in a water emulsion (Tables 47 and 48). The same type of experiment was attempted with a toxic oil, Lion Herbicidal Oil number 6, as a carrier for the 2,4-D. This oil killed the treated plants with or

Table 47. The effect of the application of the ester of 2,4-D in oil on translocation in carbohydrate depleted soybeans

Treatment	Wt. new growth, g.[a]
Oil only	3.76
200 µg 2,4-D in oil	1.23
200 µg 2,4-D in H_2O	3.31

[a]L.S.D.'s: Treatments - (.05) = .68
(.01) = .97

Table 48. Analysis of variance for the data in Table 47

Source	D.f.	S.s.	M.s.	"F"
Total	17	268.656		
Reps	5	2.1881	.4376	
Treatments	2	21.8071	10.9036	38.8**
Error	10	2.8074	.2807	

without the addition of 2,4-D. Another experiment was attempted using an emulsion composed of the non-phytotoxic oil, labeled phosphate solution and X-100. The treated plants contained almost no P^{32} except in the treated leaves. In all the experiments in which oils were used, the treated leaf, petiole and part of the stem were observed to acquire an oil soaked appearance. The toxic oil used alone killed the treated leaf, petiole and a portion of the stem adjacent to it, indicating that the oil spread into these tissues. These results substantiate the conclusions of Rice and Rohrbaugh (1953) that the oil crept through the plant tissues carrying the 2,4-D with it.

Loading of P^{32} in the detached blades of beet leaves

Leonard (1939) showed that sugars were transported quantitatively from darkened leaves of sugar beet plants, indicating translocation against steep gradients. Wanner (1953) found sucrose concentrated 20x in the phloem of black locust. Loomis (1955) has used the term loading to indicate such an accumulation in the phloem against a concentration gradient. Radioactive phosphorus suggested itself as a promising tool for the study of this process. Most of the petiole was removed from leaves to be treated, leaving only enough to reach a short distance into a beaker of water.

Labeled phosphate was applied by spreading a solution containing it and 0.2 per cent of X-100 over the leaf surface with a soft brush. Care was taken not to get P^{32} in the beaker of water. After an interval the treated leaf was washed in the manner described for treated soybean leaves. The leaves were quickly blotted, mounted on X-ray film and wrapped in black paper. The package was then placed at -20° F. and allowed to freeze and remain frozen during the exposure period. After an appropriate exposure the film was developed.

It was found that P^{32} was loaded rapidly into the veins of beet leaves. One and one-half hours after treatment the P^{32} was still distributed through the leaf in a random manner, except for a slight accumulation in the midrib at the base of the petiole (Figure 3-A). But by six hours loading of P^{32} into the veins was marked (Figure 3-B). These leaves were exposed to P^{32} for the periods indicated, washed, blotted dry and immediately placed in contact with the film. The six-hour exposure resulted in more absorption of P^{32} as well as more loading into the veins.

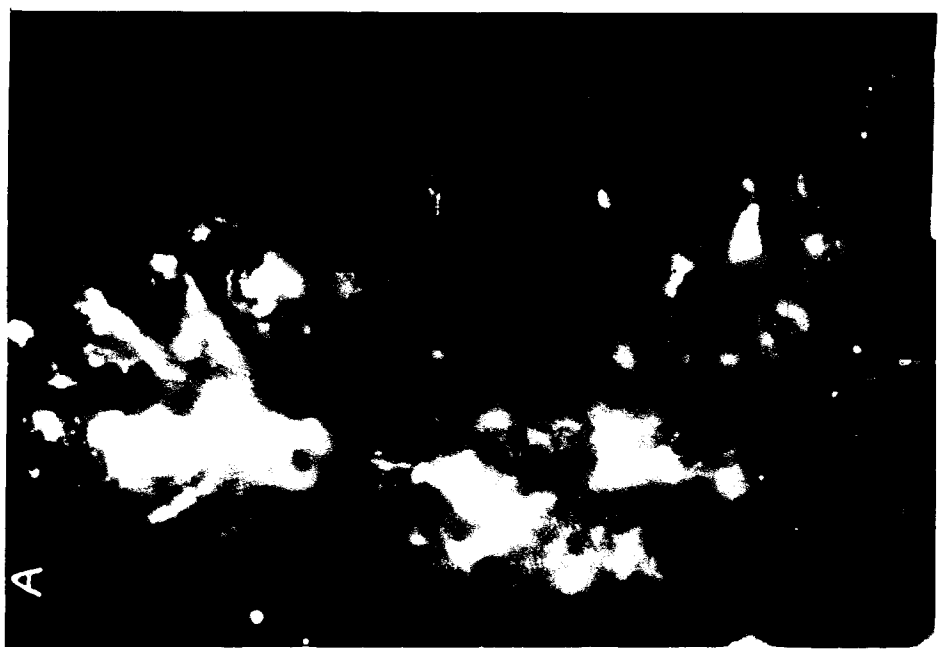
The effects of the carbohydrate supply in beet leaves on the loading of P^{32} into the veins were investigated. Leaves were shielded from light by covering them with foil and allowed to remain on the plant for 48 hours. After this period they were excised and each leaf was paired with an

Figures 3A and B. Loading of P^{32} into the veins of detached blades of sugar beet leaves.

A. Radioautograph of blade washed 1 1/2 hour after treatment. Little accumulation of P^{32} in veins.

B. Six hours after treatment much of the P^{32} was concentrated in the midrib and main veins.


B


A

unshielded leaf from the same plant. The paired leaves were both treated with labeled phosphate and the shield replaced on the one previously darkened. The leaves were held side by side under fluorescent light. After 24 hours the leaves were washed and put back in the light with the shield still on the darkened one. After another 24 hours radioautographs were made of darkened and lighted leaves.

Figure 4 depicts a leaf that was in the light, and shows a marked accumulation of P^{32} in the veins, with smaller amounts in the leaf parenchyma. In comparison, the leaf that was shielded had most of the P^{32} scattered in the parenchyma (Figure 5). The pictures in Figure 6 show similar results. The parenchmya of the leaf in the light was almost free of P^{32} (Figure 6-A) while the parenchyma of the darkened leaf contained a large portion of the total phosphorus (Figure 6-B). These pictures indicate that shielding beet leaves from light for a 96 hour period reduced the quantity of P^{32} loaded into the veins. A logical explanation for this effect is that the reduction in carbohydrate supply in the darkened leaves affected the rate of respiration and the energy available for the loading process.

If respiratory energy is used to accumulate and hold P^{32} in vein tissue against a concentration gradient, the supply of oxygen to beet leaves should affect this loading. Treated leaves were placed in two vacuum desiccators, and the air in

Figure 4. Loading of P^{32} into the veins of a sugar beet leaf blade held in light. Note heavy activity of P^{32} in all large veins and near absence in mesophyll in upper right portion of blade. Compare Figure 5.

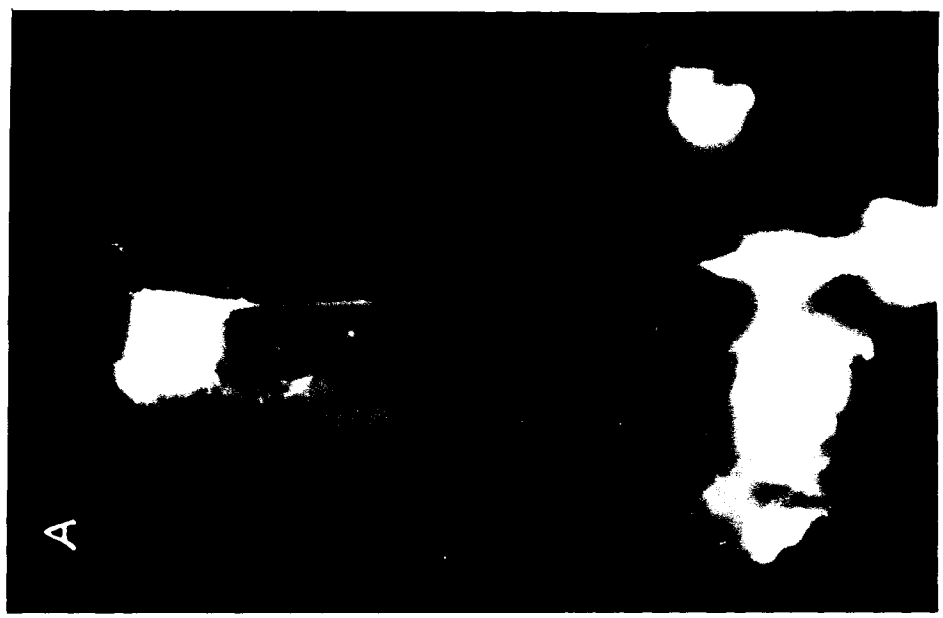
Figure 5. Greatly reduced loading of P^{32} in veins of sugar beet leaf blade held in darkness. Compare Figure 4.

Figures 6A and B. Loading of P^{32} into the veins of sugar beet leaf blades.

A. Blade in light.

B. Blade in darkness.


B


A

one desiccator replaced with nitrogen. The desiccators were alternately exhausted to a pressure of 25 mm. of Hg and refilled four times. One desiccator was filled with nitrogen each time and the other with air. The desiccators containing the treated plants were then placed in the dark for 48 hours. After this interval all the leaves were removed, washed and radioautographs made of them. Practically no P^{32} was loaded into the veins of leaves from either treatment. Attempts were made to replace the air in one desiccator by passing nitrogen through for a time then sealing it off. This experiment was also unsuccessful. The failure is attributed to the necessity of holding the leaves in darkness to prevent the liberation of oxygen in photosynthesis. The leaves had the appearance of carbohydrate depleted tissues (Figure 5).

A separation of the organic and inorganic fractions of the P^{32} absorbed by beet leaves was made using the method of Klein (1952). The leaves were treated and allowed an absorption period of six hours, then washed. After another 24 hours the leaves were ground and analyzed. Three-fourths of the added P^{32} was in the organic form (Table 49). These results suggest that the P^{32}, loaded in the phloem ready for translocation, was largely in organic compounds. Since most of the P^{32} translocated from treated soybean leaves appeared

Table 49. Total organic and inorganic fractions of P^{32} absorbed by beet leaves

Leaf	Fraction	Counts per min.	Percentage
	Total	954	
1	Inorganic	196	20.6
	Organic	758	79.4
	Total	1,789	
2	Inorganic	499	27.7
	Organic	1,293	72.3
	Total	1,420	
3	Inorganic	359	25.3
	Organic	1,061	74.7
	Total	1,933	
4	Inorganic	331	17.1
	Organic	1,602	82.9

to move in the phloem, probably the labeled phosphorus was transported in organic form.

DISCUSSION

Absorption

Absorption and translocation of foliar applications of the systemic herbicides, of which 2,4-D is the prototype, are necessary prerequisites to the control of undesired vegetation. These two processes are closely associated, and in much of the previous work with 2,4-D they have not been separated. The separation made here, based on the rapid rate of penetration of 2,4-D into leaves, is not necessarily quantitative. Some 2,4-D may have been transported from the leaf in the two-hour period allowed for absorption, but even in four hours the amount translocated was not great enough to reduce new growth of soybeans. This rate of translocation of 2,4-D is much slower than the rate found by Vernon (1951) for the translocation of labeled photosynthate. His work showed that in 45 minutes over one half of the C^{14} supplied as $C^{14}O_2$ had moved out of the treated leaf. Vernon found, however, that the rate of movement of photosynthate into the phloem was slower in plants treated with 2,4-D. The time lag between absorption of 2,4-D and its transport from the leaf suggests that the formation of a complex between 2,4-D and another molecule may be necessary before the 2,4-D is loaded into the phloem. Some time is also required for the 2,4-D

to traverse the distance from the leaf surface to the nearest phloem elements.

While the absorption of 2,4-D had been studied in some detail before this work was begun, little work had been done on the absorption of labeled phosphate by leaves. Several workers have noted that the addition of surface active agents increased the absorption of foliar applications of 2,4-D. Hauser (1955) found that both the rate of absorption and the total quantity absorbed were increased. The results were in sharp contrast when wetting agents were added to labeled phosphate. No surface active agent increased the absorption of the phosphate significantly, but two of them decreased absorption markedly. The wetting agents caused a visible spreading of the applied solution over the leaves. Staniforth and Loomis (1949) have stated that more than a decrease in surface tension of the solution is involved in the effects of wetting agents on the absorption of 2,4-D. It appears that this effect, whatever it is, does not exist with phosphate solutions. The results with Dreft and B-1956 are in agreement with those found by Swanson and Whitney (1953) for the effect of Tween 80 on the absorption of labeled phosphate. A probable explanation for the results obtained with these surfactants is that they form complexes with the phosphate which are less readily absorbed by the leaves.

The absorption of 2,4-D exhibited the temperature coeffi-

cient of a chemical reaction. Similar results were obtained by Bryan et al. (1950) and by Hauser (1955). On the other hand, the rate of absorption of labeled phosphate was found to be unaffected by increasing the temperature from 15 to 30° C. It seems that two different processes are involved, one, in the absorption of 2,4-D, chemical in nature, and the other, in phosphorus absorption, physical. Any complex formed between the phosphate and wetting agent would change the physical properties of the molecule and influence the absorption of the phosphate.

Hauser (1955) found no difference in the quantities of 2,4-D absorbed by soybeans placed in the dark for 48 hours to deplete them of carbohydrates and plants kept in the light. The work presented here shows that the addition of 5 per cent sucrose to the 2,4-D treatment did not increase the amount of 2,4-D absorbed by soybeans depleted of carbohydrates. But placing soybeans in the dark for 24 hours before treatment decreased the quantity of P^{32} absorbed. Whether or not the plants were in light before treatment was the important factor. Whether the plants were subjected to light or dark after treatment mattered very little.

The phosphate ion and 2,4-D are two quite different substances, one inorganic and the other organic in nature. There is much evidence that 2,4-D is more readily absorbed in the undissociated form. In the weakly acid solution used

(approximately pH 5) the phosphate was in the form of ions, largely $H_2PO_4^-$ and $HPO_4^=$. It might be anticipated that inorganic ions and an organic molecule penetrate into leaves by two different mechanisms. Outlines of these mechanisms may be hypothesized from the information concerning the effects that wetting agents, temperature, light and leaf carbohydrates have on the absorption of these two materials. The temperature data indicate a chemical reaction or reactions in the absorption of 2,4-D. It seems probable that this reaction occurs at a cell membrane. Phosphate appears to penetrate the leaf by the diffusion of an ion. The rate of diffusion could be influenced by the electrical charge on the leaf as well as any changes that occur in the physical properties of the molecule. If a wetting agent formed a complex with the phosphate, the larger size of this complex could cause a reduced rate of diffusion into the leaf. Waller (1925, 1929) has shown that leaves in the light are positively charged, while darkened leaves after a period of a few hours have a negative charge. He considered protoplasmic activity to be the seat of the changes involved. If the darkened leaves were negatively charged, in particular the protoplasm of them, these leaves would tend to repel the negatively charged phosphate ion and less phosphate would enter the leaves by diffusion.

Translocation

The similarities found in the translocation of 2,4-D and P^{32} are as striking as the differences in absorption of these two materials. Research by Mitchell and Brown (1946), Weaver and DeRose (1946) and many other has shown that 2,4-D applied to plant foliage is translocated in the phloem. Work by Colwell (1942), Biddulph (1944) and Swanson and Whitney (1953) has shown that P^{32} applied to plant leaves is transported from them largely, if not completely, in living tissue. It appears almost certain that the pathway is the same for 2,4-D and P^{32}, for the results of other workers as well as those presented here show that transport of the two behave in a similar manner. The large proportion of organic P^{32} in beet leaves suggests that phosphorus is transported from leaves in the organic form. This would explain why movement appears to be confined to the phloem and the similarities between the transport of 2,4-D and P^{32}.

The temperature coefficients for the translocation of 2,4-D were all calculated on the percentage reduction in growth over controls. This method was used because the reduction of soybean growth by 2,4-D follows a curve typical of the response of biological systems to toxic agents. Doubling the amount of 2,4-D in the plant does not halve the growth. The percentage reduction compared to controls gives

a better measure of the 2,4-D present in the plant than the absolute figures.

It was pointed out in the results that the temperature coefficients obtained for the translocation of 2,4-D and P^{32} are in the range encountered in chemical reactions. The effect of temperature on the viscosity of a water solution has been used to explain the effects of temperature on translocation in the phloem. The Q_{10} for such physical properties is in the neighborhood of 1.3. The Q_{10} for the change in viscosity of water between 20 and 30° C. is 1.25, and in a similar range for sucrose and glycerol solutions (Hodgman, 1952-53, pp. 1882, 1894-1895). Since the Q_{10}'s observed in the experiments with translocation were considerably greater than this, it seems likely that a chemical reaction, or series of reactions, is involved in the transport of 2,4-D and P^{32}. If this reaction, or reactions, is endothermic, metabolic energy is used to drive the process. In the range of temperature used in these experiments, an increase in temperature increases the rate of plant metabolism. This increase could supply more energy for the transport mechanism and increase the rate of translocation. This hypothesis to explain the effects of temperature on translocation in the phloem is supported by the findings of Hewett and Curtis (1948), Swanson and Bőhning (1951) and Bőhning et al. (1952). These workers found the maximum rate of translocation of

carbohydrates to be in the range of 20-30° C. Increasing the temperature above this range, as well as decreasing it, slowed the translocation of carbohydrates. This response is typical of enzymatically catalyzed reactions.

The effects of the carbohydrate supply in leaves could also be on the rate of metabolism and the supply of energy to drive the transport mechanism. On the other hand, the carbohydrate supply could be important in the transport of 2,4-D and P^{32} because it creates a concentration gradient from the leaves toward other tissues. The lack of stimulation of the transport of P^{32} by sucrose applied below it suggests that there was a strong polar transport of sucrose from the leaves. The effects of oil as a carrier for 2,4-D on translocation from carbohydrate depleted leaves probably was not related to the normal transport mechanism. The oil penetrated the plant tissues and appeared to carry the 2,4-D with it mechanically. From the experiments performed, one is unable to determine whether the supply of carbohydrates in the leaves is important in the transport of 2,4-D and P^{32} because it supplies metabolic substrates, creates an osmotic gradient from the leaves to other organs, or has other effects.

The experiments with the loading of P^{32} into the veins of excised beet leaves show where metabolic energy may be used in translocation. It appeared that applied P^{32} was

loaded into the veins of excised beet leaf blades in spite of the fact that it could not be transported from them. This loading was dependent upon the carbohydrate level of the leaf blade. Metabolic energy was undoubtedly required to load the P^{32} into the vein tissue and hold it there against a concentration gradient. More work is needed to determine the nature of this loading process and whether or not it commonly occurs in plant leaves. One place where more work is needed is with the effects of oxygen supply on loading in leaves adequately supplied with carbohydrates. Elucidation of this loading process would shed some light on the mechanism of translocation of solutes in the phloem.

SUMMARY AND CONCLUSIONS

Movement of 2,4-D from soybean leaves was found to lag behind penetration of the herbicide by two hours or more. This difference in rate constituted the basis for studies of absorption and translocation as independent processes.

Radioactive phosphorus was distributed throughout soybeans one week after application, with much of the translocated P^{32} in portions which had developed since treatment. Only 16 per cent of the P^{32} applied was absorbed by treated leaves and only about one-third of this was translocated from them.

None of five wetting agents tested caused a statistically significant increase in the absorption of labeled phosphate, but two of them, Dreft and B-1956, significantly decreased absorption in the two experiments where 20 µl of total solution was used. These findings are in sharp contrast to the results of other workers who have found surface active agents to increase the absorption of 2,4-D.

The addition of 5 per cent sucrose to 2,4-D solutions applied to carbohydrate depleted soybean leaves had little effect on the absorption of this herbicide. In contrast, plants placed in the dark for 24 hours before treatment absorbed less P^{32} than lighted plants. Whether the plants were in the light or dark after treatment had little effect

on the absorption of P^{32}. The effect is assumed to have been due to electrical charge differences rather than to carbohydrate depletion.

The absorption of 2,4-D by soybean leaves exhibited a temperature coefficient of approximately 2, while the rate of absorption of P^{32} at 15° C. differed little from the rate at 30° C. Absorption of 2,4-D by soybean roots in cultural solutions was found to have a Q_{10} near 1. The results of these and the other experiments are discussed in terms of possible mechanisms.

The amounts of carbohydrates in soybean leaves influenced the rates of translocation of 2,4-D and P^{32} from them. Sucrose applied above, or mixed with the 2,4-D or P^{32}, increased translocation from carbohydrate depleted leaves. When sucrose was applied below the P^{32}, translocation from the treated leaf was not affected.

When 2,4-D was applied in a non-phytotoxic oil it was shown to move from carbohydrate depleted leaves, while little moved from similar leaves treated with 2,4-D using water as the carrier. Mechanical movement rather than translocation in the phloem was apparently responsible.

Temperature was found to be the limiting factor in the translocation of both 2,4-D and P^{32} in the range of 15 to 30° C. The results of these and the previous experiments with translocation are discussed in terms of the nature of

the mechanism involved.

Radioautographs demonstrated that P^{32} was accumulated in the veins of excised beet leaf blades when spread over them, even though movement from the leaves was absent. The level of carbohydrates in the leaves was found to influence the quantity of P^{32} loaded into the veins. These results are evidence for a loading operation dependent upon respiratory energy as the first step in translocation.

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